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Abstracts

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^{23}Na-NMR and metabolic studies of free radical injury in renal hypoxia. *M. Cross, P.A. Stewart-Richardson, G.J. Cowin, J. Westhuyzen, S.J. Fleming, and Z.H. Endre, University of Queensland Departments of Medicine and Renal Medicine, Royal Brisbane Hospital, Herston, Australia.* Oxygen-derived free radicals (OFR) are implicated in ischemic acute renal failure (ARF). To assess this in the isolated perfused rat kidney (IPRK) we used the hydroxyl radical scavengers dimethylthiourea (DMTU) and dimethyl sulfoxide (DMSO) during a 30 minute period of hypoxia and compared with controls: changes in renal function, accumulation of renal Na measured by ^{23}Na-NMR, adenine nucleotides, glutathione and lipid peroxide metabolite levels. Control kidneys were perfused for 90 minutes. High flow hypoxia was induced at 60 minutes by switching to N_2/CO_2:95%/5%. DMSO and DMTU, both 15 mM, were added to the perfusate 15 minutes prior to the start of hypoxia. ^{23}Na-NMR spectra were collected throughout and averaged over 2 minute intervals. After 90 minutes perfusion kidneys were freeze-clamped and assayed for metabolites. Hypoxia caused a rapid decline in renal function, with reduced GFR, and increased renal vascular resistance, FENa and FEK. Hypoxia of 30 minutes produced an immediate rise in intracellular Na to ~130% of baseline and marked decreases in ATP (0.89 ± 0.25 vs. 0.19 ± 0.14 mM, SD, $N = 6$), total adenine nucleotide levels (1.44 ± 0.23 vs. 0.68 ± 0.34 mM, $N = 5$), energy charge (0.77 ± 0.06 vs. 0.45 ± 0.07, $N = 5$), and reduced glutathione content (1.57 ± 0.35 vs. 0.76 ± 0.35 mM, $N = 6$). In contrast, the levels of oxidized glutathione and lipid peroxide metabolites were unchanged following hypoxia. Scavengers of OFR did not prevent the decline in kidney function or alter the pattern of metabolite changes. OFR scavengers significantly reduced the increase in total renal sodium following hypoxia. This suggests a role for OFR-injury during the induction of injury in this model, where high flow persists with reduced Po_2, providing a continuous source of oxygen for OFR formation and illustrates the utility of ^{23}Na NMR in the dynamic monitoring of renal metabolic status in the intact kidney.

Low human kidney osmoprotectant levels are matched by low sodium and urea levels. *S. Chambers, P. Sizeland, M. Lever, L. Bason, R. Robson, Departments of Nephrology, Infectious Diseases and Biochemistry, Christchurch Hospital, New Zealand.* We have previously shown the presence of osmotically active organic solutes or "osmoprotectants" in a variety of mammalian kidneys, including humans. The levels in the human inner medulla were much lower than other mammalian kidneys analyzed. In this study the osmoprotectants glycine betaine (GB), myoinositol (MI), sorbitol (S) and glycerophosphocholine (GPC) were measured and compared with sodium and urea in human and rabbit inner medulla tissue samples. Concentrations (mmol/kg tissue) are shown in the table (mean SEM).

	Rabbits ($N = 8$)	Human ($N = 11$)
GB	64.3 (4.8)	2.7 (0.4)
MI	17.8 (1.3)	15.1 (1.3)
S	36.0 (6.8)	1.4 (0.3)
GPC	10.5 (0.7)	3.2 (0.5)
Sodium	140 (4.8)	97 (3.5)
Urea	245 (18)	39 (4.7)

In humans the low concentrations of osmoprotectants were accompanied by low sodium and urea concentrations. Significant correlations were found within and across the species between urea and GPC ($r = 0.74$, $P = 0.01$), and urea and total methylamines ($r = 0.95$, $P < 0.001$). This study confirms that the lower concentrations of osmoprotectants in human kidneys are matched by low concentrations of urea and sodium and provides evidence of a physiological relationship between the osmoprotectants and other solutes.

Effect of angiotensin converting enzyme inhibitors on plasma erythropoietin concentration in healthy volunteers. *M.C. Pratt, N.J. Lewis-Barned, R.J. Walker, R.R. Bailey, B.I. Shand, J. Livesey, Department of Medicine, Otago Medical School, Dunedin, Department of Nephrology, Christchurch Hospital, Christchurch and Department of Endocrinology, The Princess Margaret Hospital, Christchurch, New Zealand.* Angiotensin II is necessary for erythropoietin (EPO) formation. Recently it has been reported that angiotensin converting enzyme (ACE) inhibitors may cause a significant lowering of the hemoglobin concentration in patients with renal insufficiency. This is thought to be due to the ACE inhibitor affecting EPO synthesis and/or release. This single blind, cross-over study investigated the effects of low-dose captopril and enalapril on plasma EPO concentration, hemoglobin concentration and red blood cell count in 10 healthy volunteers. After 28 days treatment, there was a significant fall in mean plasma EPO concentration evident with both drugs (see Table). An insignificant, downward trend in the hemoglobin concentration and red blood cell count was found. EPO concentrations returned to baseline after stopping the ACE inhibitor.

		Day 0	Day 28	*P*
EPO *mU/ml*	Captopril	11.4 (1.2)	9.0 (1.4)	<0.05
	Enalapril	11.8 (0.9)	9.6 (0.8)	<0.05
Hemoglobin *g/liter*	Captopril	140.8 (4.6)	138.1 (6.0)	NS
	Enalapril	142.6 (5.2)	140.4 (4.8)	NS
Red blood cell count *10^{12}/liter*	Captopril	4.98 (0.14)	4.84 (0.16)	NS
	Enalapril	4.99 (0.12)	4.91 (0.14)	NS

This study has shown that ACE inhibitors can significantly decrease EPO synthesis and/or release, presumably by inhibition of angiotensin II production. This could be important in patients with renal failure, renal transplantation or other chronic conditions with an associated anemia. Hematological parameters should be monitored in such patients when they are treated with an ACE inhibitor.

Effect of neutral endopeptidase inhibition by oral SCH34826 in the rat remnant kidney model. *K. Jandeleit, M. Kanazawa, D. Casley, C.I. Johnston, and B. Jackson, University of Melbourne Department of Medicine, Austin Hospital, Heidelberg, Victoria, Australia.* Progressive renal failure is characterized by disturbed vasoactive peptide status, including alterations in the renin-angiotensin and atrial natriuretic peptide (ANP) systems. We have studied the effects of SCH34826, an inhibitor of neutral endopeptidase (EC 3.4.24.11), in the rat remnant kidney model of renal failure. Renal neutral endopeptidase is located principally in the renal tubular brush border where it acts to

degrade ANP. Four weeks after 1⅞ subtotal nephrectomy SCH34826 was gavaged for three days (90 mg/kg twice daily) in adult rats ($N = 7$) and changes compared with placebo ($N = 5$). Renal functional parameters, and systemic hemodynamic changes were assessed in awake rats. Tissue neutral endopeptidase was assessed by radioligand binding in tissue sections. Renal neutral endopeptidase was inhibited by 60%. This was associated with a 230% rise in urinary ANP, a 56% rise in urinary sodium and a 26% rise in urinary protein excretion (all $P < 0.05$). These changes were not observed in the placebo group. Glomerular filtration rate (^{99m}TcDTPA clearance), systemic blood pressure, plasma ANP concentration, plasma and urinary cGMP and plasma renin and angiotensin II were not altered by treatment with SCH34826 or placebo. These results suggest chronic administration of SCH34826 in established renal failure inhibits renal neutral endopeptidase and has intrarenal effects modulating natriuresis, without effects on systemic hemodynamics or glomerular filtration rate.

The effect of felodipine on the proximal tubule. *T.O. Morgan and D. Thomas, Physiology Department, Melbourne University, Parkville, Australia.* Felodipine, a dihydropyridine slow calcium channel blocking drug, produces a diuresis and natriuresis at doses that do not affect blood pressure (BP), RBF, or GFR, suggesting direct decreases in renal tubular reabsorption. We have used shrinking split-drop micropuncture to determine the effect of felodipine on proximal tubular reabsorption. Male Sprague-Dawley rats (250–300 g/body wt) were anesthetized with inactin. The trachea, carotid artery, and jugular vein were cannulated for infusion of saline at a rate of 2.34 ml/hr. The left kidney was prepared for micropuncture. After two hours, mid-proximal fluid uptake (Jv) was measured using shrinking split-drop micropuncture. Intraluminal felodipine at a concentration of 50 μg/ml decreased Jv by 24% ($P < 0.05$, paired t-test; $N = 7$). In a separate series of experiments, BP and proximal tubular reabsorption were measured before and 40 minutes after intravenous infusion of felodipine at a rate of 500 μg/kg/min. BP decreased from 115 to 102 mm Hg ($P < 0.001$, paired t-test; $N = 6$). Proximal tubular reabsorption decreased from a control value of 2.57 ± 0.2 to $2.26 \pm 0.2 \times 10^{-4}$ mm^{3}mm^{-2}s^{-1} ($P < 0.05$, paired t-test; $N = 6$). There was no change in the time in the control group. Dibona has provided evidence for a direct effect of felodipine on the distal tubule. The results of the present study demonstrate that felodipine inhibits proximal as well as distal tubular reabsorption and may thus inhibit transport all along the nephron.

Severe hyponatremia and atrial natriuretic peptide. *Louise M. Burrell, J.M. Palmer and P.H. Baylis, Department of Medicine, University of Newcastle upon Tyne, United Kingdom, introduced by C.I. Johnston, Department of Medicine, Austin Hospital, Heidelberg, Victoria, Australia.* The pathogenesis of hyponatremia (HX) remains debated, and to date few studies have investigated the possible role of atrial natriuretic peptide (ANP) in this disorder. Patients with severe HX (plasma sodium <125 mmol/liter, $N = 45$), and age, sex and condition matched normonatremic controls (NX, $N = 45$) were studied prospectively. Patients were classified according to clinical assessment of extracellular fluid volume as hypervolemic, normovolemic or hypovolemic. Samples were obtained after an overnight fast for the measurement of plasma sodium (pNa), plasma osmolality (pOsm), plasma ANP (pANP), plasma vasopressin (pVp), renin and aldosterone. Results are expressed as mean ± SEM, and compared using unpaired t-test.

Hypervolemia	HX ($N = 20$)	NX ($N = 20$)	P
pNa *mmol/liter*	118 ± 1.4	137 ± 1.1	<0.001
pANP *pmol/liter*	67.5 ± 19.5	45.1 ± 8.6	NS
pVp *pmol/liter*	2.9 ± 0.5	2.2 ± 0.5	NS
Normovolemia	HX ($N = 25$)	NX ($N = 25$)	P
pNa *mmol/liter*	121 ± 1.1	138 ± 0.4	<0.001
pANP *pmol/liter*	17.8 ± 2.7	5.6 ± 1.0	<0.001
pVp *pmol/liter*	2.8 ± 0.9	0.6 ± 0.1	<0.01

All patients with hyponatremia had detectable or elevated plasma vasopressin despite hypoosmolality. Plasma ANP concentrations were significantly elevated in patients with normovolemic hyponatremia, but not in those with hypervolemic hyponatremia. The mechanisms underlying the secretion of both vasopressin and ANP in normovolemic hyponatremia are unclear. This work was supported by the MRC, United Kingdom.

Machine learning applied to the diagnosis of glomerular disease. *J.W.M. Agar and G.I. Webb, Department of Medicine, Geelong Hospital, and Department of Computing and Mathematics, Deakin University, Geelong, Victoria, Australia.* Though renal biopsy is unchallenged as the diagnostic gold-standard for most renal diseases, in circumstances where biopsy is contraindicated or delayed, suitably reliable non-invasive techniques would be of significant clinical benefit. Applying machine learning strategies, we have developed expert systems (ES) which, in turn, have induced diagnostic criteria from a database of 284 consecutive biopsies (31 separate diagnoses), each comprising 38 clinical variables. Two different types of ES were developed. The first analyzed which of 31 diagnoses was present for each individual case while the second analyzed a case and determined whether or not one particular diagnosis was present. Eighty percent of cases were randomly selected to "train" the ES to develop diagnostic rules, which were then applied to the remaining 20% of cases. One hundred of each type of ES were developed, each time using a different random selection of training cases. On average, the first type of ES correctly diagnosed 53.69% cases compared to 3% expected random diagnosis and 48.31% for 4 expert clinicians given the same data. As 22/31 diagnoses comprised <10 cases each and thus limited the diagnostic accuracy, these results are highly significant. If the ES examined single conditions and was applied only where >15 cases of each diagnosis were present, the total accuracy of diagnosis was: microscopic polyarteritis 95.37%, minimal lesion nephrotic syndrome 96.50%, IgA nephropathy 81.26%, lupus nephritis 96.27%, focal glomerulosclerosis 92.06%, membranous nephropathy 92.56%, mesangial (non-IgA) proliferative GN 92.56%. We believe this pilot project has great promise, particularly with database expansion, and conclude machine learning may provide a useful diagnostic adjunct to renal biopsy in glomerular disease.

Prognosis for spinal cord injury and disease in end-stage renal failure—Australia, 1970–1991. *G.M. Patrick, J.F. Mahony, A.P.S. Disney, Department of Renal Medicine, Royal North Shore Hospital, Sydney, 2065 and ANZDATA Registry, Australia.* A survey of participating renal units for patients with spinal cord injury and disease was conducted by questionnaire in 1988 and 1990. Information regarding treatment and outcome was obtained from the ANZDATA Registry with the units' consent. Twenty-two paraplegic patients (19M and 3F) and 2 quadriplegic patients (2M) were identified in the period 1970–1991 (group A: mean age 38, range 12–65, years); 25 patients with spina bifida (19M and 6F) were identified in the same period (Group B: mean age 28, range 18–48, years). Initial treatments in Group A and Group B, respectively were: hospital HD (54% and 84%), hospital PD (25% and 8%), hospital/satellite PD (21% and 4%). The major treatments within the study period (Group A: 1631 months; Group B: 1949 months) were hospital HD (37.7% and 26.6% respectively), satellite HD (1.5% and 9.0%), home HD (33.8% and 30.5%), CAPD (7.5% and 1.3%) and transplantation (16.0% and 32.4%). Transplantation was performed in 8 patients in Group A (1 living-related and 8 cadaveric grafts) and in 13 patients in Group B (2 living-related and 11 cadaveric grafts). One paraplegic patient received 2 cadaveric transplants. Rehabilitation and quality of life data, to the time of death or the last return, were jointly assessed from the Registry on a scale of 2 to 7, a higher score being associated with a greater degree of disability. Of all patients in Group A 16.6% had a score of 2 (cf. 72% in Group B) and 29% a score of 7 (cf. 0% in Group B). Cumulative survival for Groups A and B at 1, 5 and 10 years was 91.5% vs. 100%, 71.3% vs. 100%, and 53.2% vs. 84.6%, respectively. Causes of death included myocardial infarction, sepsis, and dialysis dementia. In the paraplegic group 2 patients withdrew from treatment. The prognosis for patients with spinal cord injury and disease and end-stage renal failure is good in the long-term.

Uremic skeletal muscle metabolism: The effect of erythropoietin (EPO). *C.H. Thompson, D.J. Taylor, G.J. Kemp, J.G.G. Ledingham, B. Rajagopalan, G.K. Radda, MRC Biochemical and Clinical Magnetic Resonance Unit, John Radcliffe Hospital, Oxford, United Kingdom.* Fatigue and lethargy are common in end-stage renal failure and have

been attributed to both anemia and intrinsic metabolic myopathy. To distinguish these, we studied patients on hemodialysis before ($N = 7$) and after ($N = 4$) the hemoglobin (Hb, g/dl) rose with EPO therapy. Using ^{31}P magnetic resonance spectroscopy, we studied forearm muscle at rest, during ≤12 minutes aerobic exercise and in recovery. This allowed calculation of muscle phosphate (as Pi/ATP) at rest, the rates of phosphocreatine (P_{Cr}) depletion (Q, mM/min) and estimated lactic acid production (L, mM/min) on starting exercise, the end-exercise (ADP) (μM) (the drive for ATP production) and, after exercise, the initial rate of P_{Cr} recovery (R, mM/min, a measure of mitochondrial ATP synthesis).

	Rest		Exercise				Recovery
	Hb	Pi/ATP	L	Q	Duration	(ADP)	R
Control	13.5	0.32	1.3	8	12	30	21
Uremic	8.0[a]	0.45[a]	10[a]	22[a]	6[a]	70[a]	18[a]
On EPO	11.5[b]	0.44[a]	3.4[b]	9[b]	6.5[a]	23[b]	13[a]

$P < 0.05$; [a] cf control patients, [b] cf uremic patients pre-EPO

The rapid acidification and P_{Cr} depletion upon exercise, with near-normal P_{Cr} recovery on account of high ADP, all suggest partially-compensated mitochondrial dysfunction. Almost all these were normalized by EPO, suggesting that reduced mitochondrial O_2 delivery contributes to uremic myopathy. Exercise duration did not improve, implying some residual metabolic dysfunction. This may be related to the incomplete restoration of Hb or, more likely, to an abnormality of uremic cell metabolism related to ion transport or elevated Pi/ATP persisting after EPO.

Randomized controlled trial of cyclophosphamide (C), warfarin (W) and dipyridamole (D) in idiopathic membranous glomerulonephritis (MGN). *B.F. Murphy, I. McDonald, K.F. Fairley, P.S. Kincaid-Smith, Departments of Nephrology, St. Vincent's Hospital, Fitzroy, and The Royal Melbourne Hospital, Australia.* A previous trial of C, W, and D in MGN demonstrated marked reduction in proteinuria following 3 years therapy.[1] C rarely causes sterility if used for 6 months.[2] Accordingly, we performed a trial where the treatment consisted of 6 months C and two years W and D. Forty patients with idiopathic MGN were randomized to receive either no treatment or this regime. A significantly greater ($P < 0.05$) reduction in urine protein excretion was seen at 6, 12, 18 and 24 months in the treatment group. Plasma albumin was also significantly higher in the treatment group at 18 and 24 months ($P < 0.05$). There was no significant deterioration in renal function in either group during the two years of the trial. As progressive deterioration in renal function in MGN is associated with persistent heavy proteinuria and does not occur in the absence of the nephrotic syndrome,[3] these results suggest a beneficial effect of treatment. Such benefit would be expected to result in better preservation of renal function over longer term follow-up.

Lymphokine production by glomerular T cells in anti-GBM glomerulonephritis in mice. *P.G. Tipping, J. Demelis and S.R. Holdsworth, Monash University Department of Medicine, Monash Medical Centre, Clayton, Australia.* T cell participation has been demonstrated in proliferative glomerulonephritis (GN) in a number of species including humans, rats and rabbits. The functional role of T cells in GN is unknown. In order to study lymphokine production by T cells, a murine model of anti-GBM GN was developed. GN was initiated in mice presensitized to sheep globulin, by an intravenous dose of sheep anti-murine GBM globulin. This reliably produced proteinuria (day 5, 2.87 ± 0.59 mg/18 hr, normal 1.02 ± 0.27 mg/18 hr) and a proliferative GN with prominent glomerular macrophage infiltration. A significant infiltrate of CD3 and CD4 positive T cells was present in glomeruli from day 1 (CD3 positive T cells 2.9 ± 0.8 cells/glomerular cross section (c/gcs), normal 0.3 ± 0.2 c/gcs). Lymphokine production by isolated glomeruli in culture was assessed using specific sensitive ELISA's for interleukin 4 (IL-4) and interferon (IFNγ). IL-4 production was significantly increased on day 1 (33 ± 9 pg/10^3 gloms/24 hr) and day 3 (63 ± 16 pg/10^3 gloms/24 hr compared to normal (9 ± 9 pg/10^3 glom/24 hr). IL-4 production was not increased on day 5. In contrast IFNγ production was detectable only on day 5 (275 ± 176 pg/10^3 glom/24 hr). These studies provide evidence for a functional role of T cells in GN via lymphokine production and demonstrate a reciprocal relationship between IL-4 and IFN production by glomerular T cells.

Heavy and light chain variable regions of a lymphocytotoxic human monoclonal autoantibody are encoded within the germ line. *D.A. Power, C. Cunningham, B.K. Weber, I. Al Muzairai, P. Grabowski, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Australia.* Sera from patients undergoing dialysis commonly contain IgM autoantibodies directed to surface antigens on lymphocytes. Apart from causing confusion in interpretation of the cross match test, they appear to have little function, although they do seem to occur following viral infections. In a search for human antibodies directed to HLA antigens, we have transformed B lymphocytes from a highly-sensitized dialysis patient using Epstein-Barr virus and subsequently fused transformed lymphocytes secreting cytotoxic antibodies with the mouse-human heteromyeloma SBC-H_2O. One of the lines obtained, FWE, secretes an IgMκ autoantibody which lyses T and B lymphocytes from the original cell donor and all normal individuals as well as a variety of hematopoietic cell lines. This antibody could be removed by absorption with fetal but not adult erythrocytes, suggesting that it was directed to the fetal blood group antigen i. The variable regions of this antibody were then sequenced using cDNA obtained by reverse transcription of total cellular RNA. The variable regions were amplified by PCR using specific primers and cloned into M13mp18. The V region genes of both heavy and light chains and the J_H chain were found to be encoded by known germ-line sequences (that is, unmodified gene sequences in genomic DNA). For the heavy chain these were members of the V_HIV and J_H5 families, respectively; both families are known to be used frequently in early life when the repertoire of the immune response is being formed. These data suggest that autoantibodies in sera from potential transplant recipients are a normal part of the antibody repertoire whose synthesis is increased at certain times by viral infection.

The influence of TGF-β_1 on fibronectin synthesis and IIICS splice patterns in cultured human mesangial cells. *N.G. McKay, N.E. Haites, D.A. Power, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Victoria, Australia.* Fibronectin is one of the glycoproteins which accumulates within the mesangium in IgA nephropathy. Since there is evidence that transforming growth factor β (TGF-β) is involved in the pathogenesis of experimental mesangial proliferative glomerulonephritis, we have determined the effect of TGF-β_1 on fibronectin synthesis by cultured human mesangial cells. In both cycling and growth-arrested mesangial cells, fibronectin mRNA detected by Northern blots increased in cells stimulated with TGF-β_1 relative to controls over a 24 hour period. Study of fibronectin secretion into the extracellular matrix (ECM) and culture supernatant by ELISA, Western blotting and ^{35}S-methionine labeling showed that fibronectin synthesis was increased, but that all of the increase was deposited within the deoxycholate-insoluble ECM. One potential explanation for this pattern was considered to be an alteration in the mRNA splice pattern of the IIICS region of fibronectin which contains two cell-binding domains. Oligonucleotide primers were constructed, therefore, flanking the IIICS region of the fibronectin gene and mesangial cell cDNA amplified by polymerase chain reaction. Four products (V0, V64, V89 and V120) were obtained corresponding to 4/5 splice variants known to occur in this region. The dominant species was V0, where the whole IIICS region is excised. There was, however, no alteration in the cells stimulated with TGF-β_1 so the explanation for preferential incorporation of fibronectin into the extracellular matrix following stimulation of mesangial cells with TGF-β_1 remains obscure.

C4 null phenotype: A simple method for distinguishing point mutations from gene deletions. *J.F. Knight, G. Fanning, M.C. Falk, L.P. Roy, Renal Research Laboratories, The Children's Hospital Camperdown,*

[1] *Proc 2nd Asian Pacific Cong Nephrol* 164–172, 1983
[2] *Lancet* i:568–569, 1972
[3] *Clinical Nephrol* 30:175–181, 1988

NSW, Australia. Both homozygous and heterozygous C4 null phenotype have been associated with an increased risk of autoimmune disease. There has been controversy as to whether reduced levels of plasma C4 contribute directly to disease pathogenesis or whether the association reflects linkage with other genes in the major histocompatibility complex (MHC). The null phenotypes identifiable by electrophoresis and immunostaining of plasma correspond to a variety of abnormalities at the DNA level. Some C4 null individuals have deletions of the entire C4A or C4B genes, while others have an intact gene which is not expressed (presumably a point mutation). We have used the polymerase chain reaction (PCR) to amplify a 550 bp fragment of the genes for C4. The primers used hybridize with both the C4A and the C4B genes, and span the region coding for amino acids 1091–1206, which includes the isotypic residues which differentiate C4A from C4B. Digestion of the PCR product with the restriction enzyme NIa IV produces two bands of 325 and 290 bp. Our studies of C4 null phenotype in IgA nephropathy have led to the identification of 67 individuals with C4 null phenotype, of whom 33 have IgA nephropathy. We have submitted DNA collected from these individuals to this PCR analysis. The 290 bp band was absent in 1 of 7 individuals with homozygous C4A null phenotype and reduced to 50% intensity in another 3 of the 7. The 290 bp band was also reduced in intensity in 17/32 with C4A heterozygous null phenotype. The 325 bp band was absent in 9/11 individuals with homozyogus C4B null phenotype and reduced in intensity in 2/17 individuals with C4B heterozyogous null phenotype. The bands were both present at full intensity in another 78 individuals who did not show any C4 null phenotype at the protein level. These results suggest that the 290 bp band corresponds to the C4A gene, and the 325 bp band corresponds to the C4B gene. Absence or reduced intensity of a band suggests that the C4 null phenotype is due to a homozygous or heterozygous gene deletion, respectively. In those individuals with null protein phenotype but a normal PCR study failure of gene expression is probably due to point mutation. This method will be useful in determining whether the associations between autoimmune disease and C4 null phenotype are due to gene deletions, or point mutations, or both; the latter would imply that the association was due to the reduced level of plasma C4 rather than to linkage disequilibrium with other genes in the MHC.

Transforming growth factor-β (TGFβ) production by rat mesangial cells. *D.J. Nikolic-Paterson, H.C. Ramm, Y. Yu, and R.C. Atkins, Department of Nephrology, Monash Medical Center, Clayton, Victoria, Australia.* TGFβ is a multifunctional cytokine which has both anti-inflammatory and prosclerosing activities. Cultured glomerular mesangial cells have been shown to respond to exogenous TGFβ by decreased proliferation and increased synthesis of extracellular matrix components. Also, glomerular TGFβ production has been shown to be involved in experimental glomerulosclerosis. In this study, the potential of mesangial cells to make TGFβ was examined. Northern blot analysis of total cellular RNA using a rat TGFβ1 cDNA probe revealed expression of a single 2.5 kb mRNA species by a cloned rat mesangial cell line (1097). TGFβ1 expression was observed under conditions of serum-starvation (0.5% FCS for 3 days), rapid growth and confluency. TGFβ protein production by rat mesangial cells cultured on glass slides was detected with a rabbit anti-porcine TGFβ polyclonal antibody. Similarly, TGFβ1 gene expression was detected in a cloned mouse mesangial cell line (B2A2) under conditions of serum-starvation, rapid growth and confluency, and antibody labeling of cells cultured on glass slides confirmed the production of TGFβ protein. A faint TGFβ1 mRNA band could be detected with 20 μg of total cellular RNA from normal rat kidney. This gene expression was found primarily in the medulla and glomeruli. To assess the effects of proinflammatory stimuli on TGFβ production by resident glomerular cells, glomeruli from normal animals were cultured for 6 or 24 hours with IL-1α or LPS + PMA. Under these conditions, levels of TGFβ1 gene expression were equivalent or increased compared to freshly isolated glomeruli. In summary, cloned rat and mouse mesangial cells have been shown to constitutively express the TGFβ1 gene and to make the protein product. The demonstration of TGFβ1 gene expression in normal rat glomeruli and its maintenance under conditions of inflammatory stimuli indicates that this cytokine is likely to be involved in regulation of both normal glomerular function and its' response to inflammatory injury.

Prevention of glomerular injury, cytokine production and mononuclear and endothelial activation in nephrotoxic nephritis (NTN) by therapy with CD4 monoclonal antibody. *A. Tsuchida, J. Maguire, M. Sayegh, E. Milford, H.H. Salem, and W.W. Hancock, Departments of Pathology, Immunology and Medicine, Monash Medical School, Melbourne, Victoria, Australia and Immunogenetics and Transplantation, Harvard Medical School, Boston, Massachusetts, USA.* CD4+ T cells are instrumental to virtually all immune responses but their role, and that of cellular immunity in general, in the immunopathogenesis of glomerular injury is unknown. We studied the effects of CD4 mAb therapy in a rat model of accelerated NTN. Sprague-Dawley rats were preimmunized with normal rabbit serum and injected 5 days later with rabbit anti-rat NTN serum. Rats were treated with CD4 mAb (BWH 4,500 μg/kg/day, i.p.) for the 5 days pre-NTN or for 10 days post-NTN injection. Effects of CD4 mAb were evaluated by serial examination of renal function (Cr, proteinuria), histology, blood levels of TNF, IL-6, protein C (PC) free protein S (FPS), and immunoperoxidase for leukocytes, cytokines (IL-1, IL-2, IL-6, TNF, IFN-g) and activation markers (IL-2R, PCNA, thrombomodulin, ELAM-1 and ICAM-1). During the first 24 hours post-NTN, glomeruli in untreated rats showed infiltration by neutrophils, macrophages and T cells, in conjunction with upregulation of ELAM-1, ICAM-1 and Class II expression, fibrin deposition and downregulation of thrombomodulin. Glomeruli were also densely stained for IL-1, IL-2, IL-6 and TNF. Proliferation of glomerular cells occurred thereafter. Pretreatment with CD4 mAb decreased or abrogated many of these events; results at 2 weeks post-NTN injection were:

	Cr *mg/dl*	Proteinuria *mg/day*	Macrophages/ glomerular XS
Normal rats	1.5 ± 0.4	470 ± 69	1.5 ± 0.7
No Rx NTN	2.3 ± 0.2	1262 ± 105	6.5 ± 0.2
Rx 5 days pre-NTN	1.2 ± 0.3	686 ± 224	2.9 ± 0.9
Rx 10 days post-NTN	2.1 ± 0.6	484 ± 162	6.5 ± 0.1

TNF *pg/ml*	IL-6 *U/ml*	PC	FPS
		%	
2 ± 4	2 ± 1	100 ± 10	93 ± 9
117 ± 41	19 ± 13	66 ± 15	46 ± 3
10 ± 10	6 ± 2	77 ± 32	62 ± 10
77 ± 49	15 ± 6	77 ± 4	88 ± 6

In summary, development of NTN was associated with a mononuclear cell infiltrate and local production of the cytokines IL-1, TNF and IL-6, which in vitro are known to be mitogenic for mesangial cells. CD4 mAb maintained renal function, blocked development of proliferative GN, and prevented local cell activation and cytokine production, but only if therapy was begun pre-NTN injection; therapy post-NTN suppressed proteinuria but was otherwise ineffective. We conclude that: (1) CD4 mAb can be used to prevent but not reverse the cytokine production, intraglomerular coagulation and deterioration in renal function characteristic of nephrotoxic nephritis, and (2) such data provide important evidence for a pathogenic role for CD4+ cells in this model.

Suppression of rat nephrotoxic nephritis (NTN) by treatment with anti-IL-2R (CD25) monoclonal antibody (mAb). *W.W. Hancock, A. Tsuchida, J. Maguire, H.H. Salem, and J.W. Kupiec-Weglinski, Departments of Pathology, Immunology and Medicine, Monash Medical School, Melbourne Victoria, Australia, and Surgical Research Laboratory, Harvard Medical School, Boston, Massachusetts, USA.* CD25 mAb therapy has proven effective in clinical and experimental immunosuppression of allograft rejection and several autoimmune diseases. Given the increasing evidence for a role for IL-2R+ mononuclear cells (MNC) in the pathogenesis of renal dysfunction in various forms of glomerulonephritis and other renal diseases, we studied the effects of CD25 mAb therapy on development of a rat model of accelerated NTN. Sprague-Dawley rats were pre-immunized with normal rabbit serum and injected 5 days later with rabbit anti-rat NTN. Rats were treated with CD25 mAb (ART-18, 300 μg/kg/day, i.p.) for the 5 days pre-NTN

or for 10 days post-NTN injection. Effects of CD25 mAb were evaluated by serial studies of renal function (Cr, proteinuria); circulating levels of TNF, IL-6, protein C (PC) and free protein S (FPS); histology and immunohistology using mAbs to leukocytes, cytokines (IL-1, IL-2, IL-6, TNF, IFN-g) and activation antigens (IL-2R, PCNA, thrombomodulin, ELAM-1 and ICAM-1). CD25 mAb given either prior to, or following, injection of NTN serum was effective at suppressing development of glomerular injury and deterioration of renal function, although pre-treatment was superior to therapy begun post-NTN. Results of tests of renal function (creatinine, urinary protein), cytokine production, circulating levels of anti-coagulant proteins and leukocyte infiltration at 2 weeks post-NTN injection were:

	Cr *mg/dl*	Proteinuria *mg/day*	Macrophages/ glomerular XS
Normal rats	1.5 ± 0.4	470 ± 69	1.5 ± 0.7
No Rx NTN	2.3 ± 0.2	1262 ± 43	6.5 ± 0.2
Rx 5 days pre-NTN	1.4 ± 0.3	688 ± 231	2.1 ± 1.3
Rx 10 days post-NTN	1.3 ± 0.1	984 ± 32	4.3 ± 0.1

TNF *pg/ml*	IL-6 *U/ml*	PC	FPS
		%	
2 ± 4	2 ± 1	100 ± 10	93 ± 9
117 ± 64	19 ± 13	66 ± 15	46 ± 3
15 ± 3	7 ± 2	77 ± 33	78 ± 4
116 ± 41	9 ± 5	90 ± 13	72 ± 12

In summary, CD25 mAb therapy decreased glomerular MNC infiltration, local production of the mesangial mitogens IL-1, TNF and IL-6, and markedly diminished glomerular injury and proliferation. These data indicate an hitherto unsuspected role for IL-2R+ cells in this model, and suggest that CD25 mAb therapy may prove of clinical relevance to therapy during the acute phase of some forms of proliferative glomerulonephritis.

Respiratory burst cytochrome b558 and reactive oxygen species (ROS) in proteinuric passive Heymann's nephritis. *T.J. Neale, R. Ullrich, P.P. Ojha, H. Poczewski, A.J. Verhoeven, and D. Kerjaschki, Department of Medicine, Wellington School of Medicine, Institute of Pathology, Vienna, Austria, and Central Laboratory of The Netherlands Red Cross Transfusion Service.* Passive Heymann's nephritis (PHN), a complement-dependent rat model of human membranous nephropathy, is initiated by antibody with specificity for glomerular epithelial cell (GEC) membrane antigens. Although PHN is independent of inflammatory/phagocytic cells, ROS have been implicated in its pathogenesis. We used monoclonal antibodies with specificity for the two subunits of cytochrome b558, an essential component of the oxidoreductase responsible for the respiratory burst, to determine if glomeruli possess this capability. Both subunits were localized within visceral GEC by indirect IF in a punctate granular pattern. Immunogold EM revealed the cytochrome was restricted to GEC vesicles, the basal GEC cell membrane and vesicular extracellular structures adjacent to immune deposits. Immunoblotting detected the cytochrome in high concentrations in lysates of glomeruli isolated from proteinuric PHN rats, but in trace amounts, only in controls. Depletion of complement with cobra venom factor abolished the expression of the cytochrome implying biosynthetic dependence. As evidence of cytochrome functional activity, H_2O_2 was localized in proteinuric PHN glomerular capillary walls using a novel ex vivo cerium chloride perfusion EM histochemical technique. We conclude that the GEC has the enzymatic machinery to mount the respiratory burst, that this is amplified in proteinuric PHN and that ROS (H_2O_2) generated by the respiratory burst may have a causal role in the alteration of glomerular permselectivity in this model.

The effect of different antihypertensive agents on mesangial function following partial renal ablation. *J. Odum, J. Purkiss, P.F. Naish, School of Postgraduate Medicine, Keele University, Stoke-on-Trent, United Kingdom.* Differing classes of antihypertensive agents have different efficacy in preventing proteinuria and glomerulosclerosis (GS) in the rat remnant kidney model despite comparable reductions in systolic blood pressure (SBP). Reasons for this phenomenon are unclear. Increases in mesangial trafficking of macromolecules (MM) is proposed as one mediator of GS in this model. We studied mesangial kinetics in Wistar rats following either sham operation (2K) or 1 and 1/3 nephrectomy (4/3Nx) in untreated rats, and compared kinetics with rats treated with enalapril (EN) or the triple drug regimen of hydrallazine, hydrochlorthiazide and reserpine (HHR). EN prevents proteinuria and GS in this model whereas HHR does not. SBP elevation following 4/Nx was prevented by both EN and HHR. At 4 weeks post- 4/3Nx or 2K, rats underwent arterial injection with iodinated aggregated BSA (I-aggBSA) at a dose of 30 mg/100 g body weight. Groups of rats (5 at each time point) were sacrificed at 2, 4 and 8 hours post-injection and concentration of I-aggBSA in blood, heart, liver, spleen and isolated glomeruli quantified. At each time point there were no differences between any group of rats in concentration of I-aggBSA in blood, heart, liver or spleen. Mesangial kinetics were identical in 2K rats treated or untreated. Kinetics in 4/3Nx rats are shown below (mean ± SEM). Uptake of I-aggBSA is expressed as μg/mg glomeruli.

	2 Hours	4 Hours	8 Hours
2K	0.714 ± 0.08	0.738 ± 0.08	0.462 ± 0.03
4/3Nx	0.766 ± 0.13	1.336 ± 0.24[a]	0.519 ± 0.12
4/3Nx EN	0.785 ± 0.11	1.006 ± 0.07	0.932 ± 0.13[b]
4/3Nx HHR	1.159 ± 0.24	0.959 ± 0.42[c]	0.865 ± 0.25

[a] $P = 0.001$; [b] $P = 0.002$; [c] $P = 0.0001$; all vs. 2K

These results confirm that increases in mesangial uptake and clearance of MM occur in remnant glomeruli. Trafficking is reduced by EN treatment by mechanisms affecting clearance of MM from the mesangium. Increases in trafficking were maintained by HHR, a therapy associated with progressive GS.

Deoxyspergualin treatment prevents renal function impairment in experimental anti-GBM glomerulonephritis (GN). *H.Y. Lan, D.J. Nikolic-Paterson, M. Zarama-Medina, P.G. Kerr, R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* Deoxyspergualin (DOSP), a new immunosuppressant drug, has been reported to modify the course of spontaneous lupus-like glomerular lesions in susceptible mice. We have investigated the effects of DOSP-treatment in accelerated anti-GBM GN in the rat. Treatment consisted of daily intraperitoneal injection of DOSP (5 mg/kg body wt) or vehicle alone from day 0 until sacrifice. Groups of 5 animals were sacrificed at days 7, 14, and 21. Macroscopically, untreated animals developed large white kidneys, whereas kidneys from DOSP-treated animals maintained a normal appearance and weight ($P < 0.001$). Results for hematuria (HU), proteinuria (PU) and creatinine clearance (C_{Cr}) are shown below. Untreated animals developed severe hematuria, severe proteinuria, and exhibited a significant decrease in C_{Cr} at days 14 and 21. DOSP-treatment produced complete suppression of haematuria, and maintained normal renal function as assessed by C_{Cr}. However, DOSP-treatment had only a limited effect upon proteinuria, with only the day 14 group demonstrating a significant decrease. In conclusion, DOSP-treatment improved renal function and reduced renal damage in accelerated anti-GBM GN. Thus, DOSP may be a potential new drug for the treatment of human GN.

	HU *0–4+*		PU *mg/24 hours*		C_{Cr} *ml/min*	
	untreat	treat	untreat	treat	untreat	treat
Day 7	+ (2) ++ (3)	0 (5)	262 ± 55	297 ± 60	1.4 ± 0.1	1.4 ± 0.2
Day 14	++ (2) +++ (3)	0 (5)	445 ± 59	228 ± 65[a]	0.9 ± 0.1	1.4 ± 0.1[b]
Day 21	+++ (5)	0 (5)	452 ± 82	336 ± 120	0.6 ± 0.1	1.4 ± 0.1[b]

[a] $P < 0.05$, [b] $P < 0.001$, compared to untreated group

Glomerular fibrinolytic activity (GFA) in anti-GBM induced glomerulonephritis (GN) in rabbits. *J. Malliaros, P.G. Tipping, J. Wojta, and*

S.R. Holdsworth, Monash University Department of Medicine, Monash Medical Centre, Clayton, and Department of Diagnostic Haematology, Royal Melbourne Hospital, Parkville, Australia. Fibrin is an important mediator of injury in GN. Glomerular fibrin deposition (GFD), is dependent on macrophage infiltration and is associated with augmented glomerular procoagulant activity. The contribution of GFA to GFD in GN is unknown. Changes in GFA were studied in GN with marked GFD and prominent macrophage infiltration (autologous phase anti-GBM GN) and contrasted with GN without GFD or macrophage infiltration (heterologous phase anti-GBM GN) in rabbits. Total GFA was measured by a solid phase plasminogen-dependent ^{125}I fibrin lysis assay. GFA was significantly reduced in autologous GN (1.27 ± 0.88 ng fibrin lysed per 10^3 glom/2 hr), compared to normal glomeruli (57.1 ± 27.8 ng/10^3 glom/2 hr; $P < 0.05$). GFA was augmented during heterologous GN (174 ± 64 ng/10^3 glom/2 hr). Fibrin autography revealed both tissue plasminogen activator (tPA) and urokinase (uPA) activity in normal glomeruli. Both tPA and uPA were decreased in autologous GN but not in heterologous GN. Reverse fibrin autography revealed increased plasminogen activator inhibitor type 1 (PAI-1) activity in autologous GN compared to normal glomeruli and heterologous GN. PAI-1 production measured by ELISA was significantly increased in autologous GN (0.10 ± 0.03 ng/10^3 glom/24 hr) compared to normal glomeruli (0.02 ± 0.01 ng/10^3 glom/24 hr; $P < 0.05$). PAI-1 was undetectable in heterologous GN. These results demonstrate that fibrin deposition in GN is associated with decreased GFA due to decreased production of tPA and uPA and increased production of PAI-1. These changes favor fibrin accumulation in GN.

Compensatory renal hypertrophy (CRH): Cell growth and transport studied in primary proximal tubular cell culture (PPTCC). *C.A. Pollock and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, NSW, Australia.* Significant alterations in the tubular handling of Na are observed in vivo in CRH. In order to determine whether altered growth and transport characteristics persist in vitro, we measured these properties in confluent PPTCC derived from normal animals (N), and those with CRH induced by nephrectomy performed 4 to 6 weeks earlier. Enhanced growth potential was retained in the CRH cells compared with N cells, as evidenced by an increased cellular protein content (250 ± 12 vs. 151 ± 11 pg protein/cell; $P < 0.001$), and a somewhat lesser increase in cellular thymidine incorporation (6820 ± 52 vs. 5151 ± 308 cpm/10^6 cells; $P < 0.02$). Cellular Na transport (measured as ^{22}Na uptake) was increased in the CRH cells when compared with N, being 733 ± 47, 886 ± 111 and 1026 ± 147 cpm/1,000 cells in CRH cells at 1, 3 and 120 minutes, respectively, vs. 353 ± 36, 469 ± 31 and 549 ± 41 cpm/1,000 cells in N cells ($P < 0.05$ for each time point), that is, an increase in Na transport in CRH cells of 107, 88 and 87%, respectively. Administration of amiloride inhibited Na uptake in N cells by approximately 50% and completely suppressed the excess Na uptake in CRH cells to that observed in normal; that is 147 ± 33, 224 ± 91 and 174 ± 86 cpm/1,000 cells in CRH cells at 1, 3 and 120 minutes, vs. 181 ± 71, 164 ± 57 and 238 ± 85 cpm/1,000 cells in N cells (P = NS at each time point). The results suggest that enhanced tubular cell growth in CRH is maintained from the in vivo setting to the in vitro cell culture system. Increased cellular Na transport in CRH is demonstrated to be via Na-H exchange by virtue of its reversion to normal in the presence of amiloride. Taken in conjunction with data from our previous micropuncture and electron microprobe experiments, the results support the view that in established CRH in vivo, the hypertrophic response is limited by growth inhibitors which act primarily or secondarily by an alteration in epithelial transport. In vitro, the effect of these factors is removed, allowing an unrestrained stimulatory growth response, accompanied by enhanced Na transport.

Anti-endothelial cell antibodies (AECA) and their functional and histological associations in lupus nephritis. *G.J. Perry, T. Roedecker, T.M. Chan, J.S. Cameron, G. Frampton, Renal Unit, UMDS, Guy's Hospital, London, United Kingdom.* Anti-endothelial cell antibodies have been well documented in SLE. Their functional properties and histological associations are mainly unknown. Twenty-two patients with biopsy-proven lupus nephritis were studied. AECA, anticardiolipin (ACA), anti-dsDNA antibodies (DNA) and serum complement levels were measured at the time of biopsy and subsequently in remission. Protein S and von Willebrand factor, markers of endothelial cell function, were also measured. Renal biopsies were scored for active and chronic damage. AECA were present in 80% of patients biopsied and changes in AECA levels reflected disease activity to the same degree as dsDNA antibodies and C3 levels whereas C4 levels and ACA did not. Active disease was associated with elevated levels of von Willebrand factor and reduced levels of Protein S. There was no association demonstrated between AECA and histological scores of activity or chronicity. AECA reflect disease activity in a similar fashion to dsDNA antibodies. The high incidence of AECA with active lupus nephritis and their association with serological markers of endothelial cell injury indicate that they may be an important factor in the pathogenesis of lupus nephritis.

Effect of a water load on urinary excretion of osmoprotectants. *P. Sizeland, S. Chambers, M. Lever, L. Bason, R. Robson, Departments of Nephrology, Infectious Diseases and Biochemistry, Christchurch Hospital, New Zealand.* In vitro studies using cell-culture have shown a rapid response of the kidney osmoprotectant system to different osmotic conditions. We have developed sensitive assays capable of accurately measuring the osmoprotectants glycine betaine (GB) and sorbitol (S) in plasma and urine. This study was designed to measure changes in GB and S in plasma and urine in response to an acute water load (20 ml/kg) and involved healthy males. Plasma and urine samples were collected at regular and frequent intervals during the study period and subsequently analyzed for plasma GB and urine minute volume, urea, GB and S. The rate of urinary excretion of GB in 4/6 and S in 5/6 subjects changed in parallel with urinary urea excretion, with an initial increase preceding maximum urine minute volume, followed by a gradual return to baseline values. There was no change found in plasma GB concentration during the study period. We conclude that the osmoprotectants GB and S are components of a physiological and dynamic system, at least in response to an acute water diuresis.

Comparison of intravenous and oral alfacalcidol in the treatment of secondary hyperparathyroidism in hemodialysis patients. *W.T. Lee, J.F. Collins and T. Cundy, Department of Medicine, Auckland Hospital, Auckland, New Zealand.* High dose pulse intravenous alfacalcidol can suppress parathyroid hormone (PTH) levels independent of serum calcium (Ca) in haemodialysis patients with secondary hyperparathyroidism. To determine the optimal schedule of administration of alfacalcidol, we conducted a randomized prospective crossover study of high dose pulse oral and intravenous alfacalcidol on 12 hemodialysis patients on low Ca dialysate (1.2 mmol/liter). Patients received a 6 weeks course of oral alfacalcidol (4 μg 3×/week) or intravenous alfacalcidol (4 μg 3×/week) with a washout phase before they were crossed over. Levels of iPTH, ionized Ca and total Ca were obtained weekly. The alfacalcidol dose was reduced if the total Ca level exceeded 2.7 mmol/liter. There was a significant reduction in iPTH ($P < 0.001$) and a significant increase in ionized Ca levels ($P < 0.001$) with both intravenous and oral alfacalcidol treatments.

	iPTH (±SEM)		Ionized Ca (±SEM)	
Treatment	Baseline	Treatment	Baseline	Treatment
Intravenous	28.5 (±2.9)	22.0 (±3.0)	1.24 (±0.01)	1.37 (±0.04)
Oral	35.1 (±3.4)	22.0 (±2.5)	1.21 (±0.01)	1.31 (±0.02)

There was no difference in the degree of iPTH suppression between oral and intravenous alfacalcidol. With both treatments iPTH levels decreased before a rise in ionized Ca levels. The doses of alfacalcidol were reduced in 8 out of 12 intravenous treatments and in 7 out of 12 oral treatments because of hypercalcemia. High dose pulse oral alfacalcidol is as efficacious as high dose intravenous alfacalcidol in the suppression of secondary hyperparathyroidism in hemodialysis patients. This effect precedes a rise in ionized Ca levels.

VCAM-1 gene expression in rat kidney and cultured glomerular cells. *D.J. Nikolic-Paterson, Y. Yu, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* Vascular cell adhesion molecule-1 (VCAM-1) is expressed by endothelial cells following cytokine stimulation and acts as a receptor for the VLA-4 antigen expressed by activated T cells and monocytes. VCAM-1

is thought to be involved in the extravascularization of activated T cells and monocytes. Little is known about expression of adhesion molecules in normal or diseased kidney, thus we have studied VCAM-1 gene expression in the rat kidney by Northern blot hybridization using a rat cDNA probe. Expression of a single species of VCAM-1 mRNA (≈3.4 kb) was detected in total cellular RNA from normal rat kidney. VCAM-1 gene expression was most clearly detected in the medulla and in glomeruli. In some cases, a second higher molecular weight mRNA species (≈3.8 kb) was detected in the medulla. Regulation of glomerular VCAM-1 gene expression was examined in vitro. A significant increase in VCAM-1 mRNA was observed in isolated normal glomeruli following a 6 hour culture with rhum IL-1α (10 U/ml), but this was markedly reduced at 24 hours. A similar transient upregulation of glomerular VCAM-1 gene expression was seen using LPS (5 μg/ml) + PMA (1 ng/ml) stimulation. In addition to endothelial cells, one candidate for inducible glomerular VCAM-1 gene expression is the mesangial cell. Preliminary studies with a cloned rat mesangial cell line revealed VCAM-1 gene expression following a 6 hour stimulation with rhumIL-1α (10 U/ml). In conclusion, VCAM-1 is expressed in the medulla and glomeruli of the normal rat kidney. Two different forms of this molecule, which differ in their extracellular portions, may be expressed in the kidney on the basis of alternate mRNA splicing. The transient upregulation of glomerular (including mesangial cell) VCAM-1 expression by proinflammatory stimuli may be one mechanism by which leukocyte infiltration in the glomerulus occurs.

A novel human antigen (254Ag) associated with pathological and non-pathological complement C5b-9 deposits. *M. Polihronis, J. Macrae, J. Saunders, M. O'Bryan, D. Power, A. d'Apice, B.F. Murphy, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Australia.* An IgG1 monoclonal antibody (mAb 254) was raised against pathological glomerular basement membrane from a patient with membranous glomerulonephritis. 254Ag was found to co-localize exactly with SC5b-9 (components and neoantigen) in glomerular immune deposits and areas of glomerular sclerosis in a prospective study of 50 renal biopsies. 254Ag is absent from normal glomeruli but is invariably detected in the media of normal muscular arteries, the portal tracts and capsule of normal liver and the splenic capsule and trabeculae; in all of these sites it is associated with small amounts of co-localized C5b-9. Detergent treatment of tissues partially removes SC5b-9 and 254Ag, but both are resistant to treatment with high salt, acid and denaturing buffers. 254Ag was not detected in normal human plasma or zymosan activated human serum and mAb 254 does not react with purified SC5b-9 or C5b-9(m) complement complexes generated in vitro. Extraction of liver capsule by with sodium dodecyl sulphate produces a soluble preparation containing SC5b-9 and 254Ag by dot blot analysis. Preliminary western blotting data suggest that 254Ag may represent an epitope on the highly polymerized form of C9 seen in membrane bound C5b-9. As such it may be the first reagent able to distinguish membrane bound C5b-9 [C5b-9(m)] from the soluble non-lytic SC5b-9 form.

Immunogenetic studies of IgA nephropathy. *J.F. Knight, G. Ng, Y. Zhang, C.M. Artlett, M. Falk, and L.P. Roy, Renal Research Laboratories, The Children's Hospital, Camperdown, Australia.* IgA nephropathy is the most common cause of glomerulonephritis in Australia. Its reported genetic associations are with HLA B35, DR4 and DQw8, with polymorphisms of components of the complement pathway (C2, C3, Bf, C4A, C4B and C7) and with restriction fragment length polymorphisms (RFLPs) of the μ-switch, α_1-switch and α_2-switch regions of the immunoglobulin heavy chain gene. These associations are observed in some populations but not in others. As part of an ongoing study of Australians with IgA nephropathy, plasma samples and DNA extracted from peripheral blood white cells of 70 patients and 210 healthy blood donors were examined for genetic heterogeneity at some of the loci listed above. No associations were observed with C2, C3 or Bf plasma protein polymorphisms. The patient group did however show an increased incidence of C4B heterozygous null phenotype: 15/70 (21%) vs. 20/210 (9%); $P = 0.01$; $\chi^2 = 6.16$). C4B homozygous null phenotype was also increased: 6/70 (8%) vs. 7/210 (3%), but did not reach statistical significance. The gene frequency for C4B null was 0.084 in controls and 0.193 in patients ($P = 0.0007$, $\chi^2 = 11.4$). C4A heterozygous null phenotype, on the other hand, was more common in controls (67/191; 35%) than in patients (11/70; 16%): $P = 0.004$; $\chi^2 = 8.25$. C4A homozygous null phenotype was similar in both groups. The gene frequency for C4A null was 0.157 in patients and 0.351 in controls ($P = 0.026$ $\chi^2 = 4.93$. All statistical analyses were performed with Yates' correction. Southern blots of genomic DNA cut with the restriction enzyme Sst I were hybridized with a cDNA probe which has sequence homology with both the μ-switch and the α_1-switch regions of the immunoglobulin heavy chain gene. No difference was seen between patients ($N = 66$) and controls ($N = 196$) in the distribution of polymorphisms at these loci. This study demonstrates that in this group of Australian patients, IgA nephropathy is associated with an increase in C4B null phenotype and a decrease in C4A null phenotype. The other associations reported in other centers were not apparent.

Endoglin, a 180kD endothelial cell and macrophage restricted differentiation molecule. *Phillip J. O'Connell, Adrienne McKenzie, Nella Fisicaro, Steven P. Rockman, Martin J. Pearse, and Anthony J.F. d'Apice, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, and the Departments of Nephrology and Haematology, Royal Melbourne Hospital, Victoria, Australia.* Endothelial cells (EC) have been shown to play an active role in many immunological functions including hemopoesis, the homing of lymphocytes to specific organs and the migration of granulocytes and monocytes to areas of inflammation. ECs also share many of the characteristics of myelomonocytic cells including antigen processing and presentation and secretion of interleukin 1, interleukin 6, and proteases. As would be expected from the sharing of various functions, ECs and myelomonocytic cells share many surface molecules. This report describes a mAb, RMAC8, generated by immunizing Balb/c mice with cultured human umbilical vein endothelial cells (HUVE). The molecule recognized had a Mr of 180kD non-reduced, 95kD after reduction and 66 kD in its reduced and N-deglycosylated form. The pI of the reduced molecule was 4.8–5.0. Sequential immunoprecipitation studies with the mAb 44G4, which recognizes the O- and N-glycosylated homodimer endoglin, showed that both mAb recognize the same molecule on PMA stimulated U937 cells (human monocyte/macrophage cell line). The distribution of the RMAC8 recognized molecule was the same as that described for endoglin, that is, arterial and venous endothelium, myelomonocytic and pre-B leukemia cells and cell lines, however, unlike 44G4, RMAC8 also reacted weakly with monocytes and strongly with in vitro differentiated macrophages and peritoneal macrophages. This distribution of endoglin was confirmed by Northern blot analysis using a full length endoglin cDNA probe. These studies suggest that endoglin is a differentiation marker on macrophages where the epitope recognized by 44G4 is hidden.

Epidermal growth factor (EGF) production by rat mesangial cells. *D.J. Nikolic-Paterson, H.C. Ramm, T. Ootaka, N. Kraft, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* EGF production in the kidney is restricted to the luminal surface of specific tubular epithelial cells, and therefore only circulating EGF has access to the mesangium. Cultured mesangial cells have been shown to express EGF-receptors and to proliferate in response to exogenous EGF. We investigated whether cultured mesangial cells could also synthesize EGF which could act as a potential autocrine growth factor. Northern blot analysis of total cellular RNA from cloned rat and mouse mesangial cell lines (1097 and B2A2) demonstrated the expression of a single ≈5 kb EGF mRNA species. EGF gene expression was observed under conditions of serum starvation (0.5% FCS for 3 days), rapid growth, and confluency. EGF protein production by rat and mouse mesangial cells cultured on glass slides was detected with a rabbit anti-mouse EGF polyclonal antibody by the immunoperoxidase method. Also, EGF protein production by mesangial cells cultured in 96 well trays was detected with the polyclonal antibody using an ELISA technique. Furthermore, preliminary Western blot studies revealed the presence of 3 EGF species (roughly 60, 50, and 10 kD) in mesangial cell lysates. To investigate whether EGF production by mesangial cells was involved in an autocrine pathway, rabbit anti-mouse EGF antibody was added to cell lines in culture. Antibody addition was able to neutralize the stimulatory effect of exogenous mouse EGF on both cell lines, but failed to inhibit baseline proliferation. In conclusion, this study demonstrates that cultured rat and mouse mesangial cells synthesize EGF under various growth conditions. The inability of anti-EGF antibody to

inhibit mesangial cell proliferation suggests two possibilities: (1) EGF derived from mesangial cells is not processed as a functionally active molecule, or (2) the antibody is unable to compete with the mechanism of autocrine EGF utilization.

Quantitative and semi-quantitative estimation of fibronectin mRNA splice variants in cultured human mesangial cells using polymerase chain reaction. *D.A. Power, N.E. Haites, M.C. Jones, N. McKay, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Australia.* Fibronectin is a glycoprotein present in extracellular matrix (ECM), possessing three known sites for adherence to cells. Two of these can be removed by alternating splicing in the IIICS region of the molecule; the best described cell attachment site, the only one bearing an RGD sequence, lies between two further alternative splice sites, EIIIA and EIIIB. In order to study the influence of cytokines on synthesis and splicing of fibronectin by cultured human mesangial cells, methods were developed for the quantitative and semi-quantitative estimation of mRNA transcript numbers. Transcript numbers for two splice variants of fibronectin were determined quantitatively using an internal control; semi-quantitative estimation of 4 of the 5 splice variants in the IIICS region was also performed. In the quantitative assay, primers flanking the EIIIA domain were used to amplify RNA DNA hybrids by polymerase chain reaction (PCR). In order to quantitate transcript numbers, a control species was produced by excising the mid-portion of a cDNA probe for fibronectin between two sites for the restriction enzyme Xho. Using radiolabeled primers and quantitative PCR, it was found that approximately 2/3 transcripts lacked EIIIA in both cell types. The mean number of fibronecting transcripts per cell was approximately 4.6. Interassay coefficients of variation for the assay were 14% for transcripts with EIIIA and 10% for the dominant species. For semi-quantitative assessment of splice variants in the IICS region, primers flanking this region were used to amplify reverse transcribed RNA from mesangial cells and the products separated on agarose gels. The percentage of each was estimated by densitometry. These studies demonstrate the absolute or relative number of RNA splice variants in a cell can be determined using quantitative PCR.

Inhibition studies of the functional properties of neutrophil lysosomal enzymes. *Linus Chang, and Judy Savige, Department of Haematology, Heidelberg Repatriation Hospital, Banksia Street, Heidelberg West, Victoria, Australia.* Anti-neutrophil proteinase-3 (29kd), anti-Myeloperoxidase and anti-elastase are three members of a novel class of autoantibodies against lysosomal enzymes present in human neutrophil cytoplasmic granules and are associated with systemic vasculitis. Untreated cases of Wegener's granulomatosis, microscopic polyarteritis, and crescentic glomerulonephritis may result in renal failure. Immunosuppressive regimes drastically improve prognosis of these conditions which are associated with systemic vasculitis, implicating involvement of the autoantibodies in the pathogenesis of these diseases. To investigate whether functional and immunogenic properties share common epitopes, we studied the inhibitability of these enzymes by their respective antibodies. Neutrophil proteinase-3 is a serine esterase and its activity was measured by the cleavage of alpha-naphthal acetate by neutrophil granule extract. Myeloperoxidase activity was measured by two methods: peroxidation of guaiacol by hydrogen peroxide (H_2O_2), and peroxidation of monochlorodimedon by H_2O_2 in the presence of chloride ions. Elastase activity was measured by fluorimetric assay of the hydrolytic product of N-Succ ala3 amido-methyl coumarin. Inhibition of these enzymes was performed by incubating positive (assessed by Enzyme Linked Immunosorbent Assay) patient sera or immunoglobulin with the enzymes in liquid phase or first incubating sera or immunoglobulin with the respective enzymes precoated on Dynatech plates before activity assays. In all three enzymes no inhibition of catalytic activity was observed in either the liquid phase or solid phase inhibition procedure. These results suggest that catalytic and immunogenic properties of these enzymes share very little or have no common epitopes.

Production of pro-inflammatory cytokines by resident glomerular cells. *Y. Yu, D.J. Nikolic-Paterson, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* This study has investigated whether resident glomerular cells, in the absence of an inflammatory infiltrate, are capable of producing pro-inflammatory cytokines in response to specific stimuli. Glomeruli were isolated from Sprague-Dawley rat kidneys (2–4 months old) by differential seiving, cultured for 6 or 24 hours in RPMI/10% FCS with 10 U/ml recombinant human IL-1α or 5 μg/ml LPS plus 1 ng/ml PMA, and total cellular RNA prepared. Northern blot analysis failed to detect IL-1β gene expression in normal kidney or freshly isolated glomeruli. However, a 6 hour culture with IL-1α induced expression of a single IL-1β mRNA species ($\approx$1.8 kb) which was downregulated by 24 hours. LPS + PMA stimulation also caused an induction of IL-1β mRNA which was sustained at 24 hours of culture. IL-6 gene expression was not detected in fresh renal tissue, but was clearly evident in glomeruli following a 6 hour stimulation with IL-1α or LPS + PMA. In contrast to IL-1β gene expression, levels of IL-6 mRNA ($\approx$1.3 and 2.4 kb) remained elevated at 24 hours in response to both stimuli. In order to check that cytokine mRNA production was not due to contaminating blood cells, kidneys were perfused in situ with PBS. Comparable levels of both IL-1β and IL-6 mRNA species were evident in glomeruli prepared from perfused and non-perfused kidneys following in vitro stimulation. To establish whether culture alone could induce cytokine expression, glomeruli were cultured in RPMI/10% FCS, and only a very faint IL-6 mRNA signal was detected. In conclusion, resident glomerular cells have been shown to express pro-inflammatory cytokine genes in response to specific pro-inflammatory stimuli. This may be an important mechanism in the induction of glomerular leukocytic infiltration by chemotaxis and expression of adhesion molecules. Also, such cytokine production may regulate the function of other resident glomerular cells.

Function of increased ICAM-1 expression on interferon gamma (IFNg) stimulated mononuclear phagocytes. *P. Hutchinson, N. Kraft, and R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* ICAM-1 is a cell surface glycoprotein found on vascular endothelium and epithelial cells, but is only weakly expressed on resting leukocytes. It is a ligand for the LFA-1 molecule and is a critical component of T-cell activation in some immunological reactions. As IFN-g is known to increase ICAM-1 expression on some cells and to potentiate macrophage accessory activity, we examined it's effect on ICAM-1 expression on a variety of phagocytes. Addition of IFNg (1000 U/ml) led to a rapid induction of ICAM-1 (2–6 hours) on the myelomonocytic lines U937 and Rc2A with a plateau response at day one and maintained to day 3 (U937: $N = 7$, 361 $\pm$ 104%; Rc2A: $N = 6$, 338 $\pm$ 146%). The IFNg response was dose-dependent to 300 U/ml. Blood derived monocytes cultured in teflon bags for three days showed an increase in ICAM-1 of 282 $\pm$ 121% ($N = 5$), with an early response apparent at 2 hours. PHA stimulation of T-cell proliferation was increased when IFNg treated Rc2A cells were used as accessory cells compared to non-treated cells, but there was no increased blocking by anti-ICAM-1 antibody. However, the allogeneic response of the T-cells to IFNg-treated Rc2A cells was significantly greater than against untreated Rc2A cells and was inhibited by anti-ICAM-1 antibody. This may indicate specific roles for the increased ICAM-1 expression on phagocytic cells. Interleukin-1 production by both the IFNg treated and control Rc2A cells was minimal, while addition if recombinant IL-1 and IL-6 to the allogeneic response did not consistently increase the proliferation of the T-cells against the control Rc2A cells to the level of the IFNg treated cells, implying a limited role of these cytokines in the heightened response.

Maintenance of the integrity of C8 and C9 of the complement membrane attack complex in the sera of patients with SLE. *B.A. Pussell, C. Morris, L. Milis, V. Timmermans and J.A. Charlesworth, Department of Nephrology, Prince Henry Hospital, Sydney, NSW, Australia.* Activation of the complement cascade is a hallmark of systemic lupus erythematosus (SLE) with predominant depletion of classical pathway components by ongoing activation. In addition, genetic deficiencies of early components are frequently associated with SLE or SLE-like illnesses. In contrast, deficiencies of the membrane attack pathway do not have an association with SLE but with infection by intracellular microorganisms, especially Neisseria. However, involvement of the membrane attack complex (MAC) in the mediation of renal injury has been reported in both experimental animal models of glomerulonephritis and in human kidneys using immunofluorescence techniques. In order to test the integrity of the MAC in patients with SLE, we used an in vitro assay of the functional activity of C8 and C9. Sheep red blood

cells(E) coated with antibody(A) were used to activate complement in serum deficient in C8 and to form stable EAC1-7 cells. The MAC was then completed by the provision of C8 and C9 in very dilute normal or test serum and cell lysis estimated by optical density. All 15 SLE sera tested gave identical lysis to normal sera despite levels of low CH50, C3 and C4. We conclude that functional activity of C8 and C9 of the MAC of complement is retained in the sera of patients with SLE despite evidence for classical pathway activation. Such a finding has implications for the sites of complement activation in SLE and the particular complement components involved in tissue injury.

Western blot analysis of membrane antigens recognized by anti-endothelial cell antibodies (AECA) in SLE. *G.J. Perry, N. Gregson, T.M. Chan, J.S. Cameron, G. Frampton, Renal Unit, UMDS, Guy's Hospital, London, United Kingdom.* We have demonstrated that AECA occur in 35% of patients with systemic lupus erythematosus (SLE). The target epitopes have not been fully characterized. The endothelial cell line EAHy 926 (gift of Dr. C-J Edgell) was screened for the presence of the autoantigen(s) using a cell-based ELISA and compared with the standard human umbilical vein endothelial cells. The binding correlation between the two cell populations was $r = 0.72$ ($P < 0.001$). This suggested that the autoantigen(s) was present on both cells. Plasma membranes from EAHy 926 cells were prepared and used for Western blotting with AECA positive SLE sera. Multiple bands could be identified. SLE patients with and without nephritis were compared. In general, a band of approximate molecular weight 66 kD was present in both groups and was the most common band detected. Other bands could be detected which were predominantly associated with nephritis. This provides further evidence for the autoantigens recognised by AECA and suggests that some antigens may be relevant in the pathogenesis of lupus nephritis.

Altered in vitro lymphocyte response in childhood nephrotic syndrome. *I.K. Hewitt, A.K. House, J.M. Potter, B.F. Kinnear, Department of Nephrology, Princess Margaret Hospital for Children, Perth, W.A., Australia.* The immune system, and disturbed T lymphocyte function in particular has been implicated in the pathogenesis of childhood idiopathic nephrotic syndrome (INS). As this disorder is commonly responsive to steroid therapy, we set out to determine whether in vitro suppression of lymphocyte blastogenic response to the mitogen PHA could predict the clinical outcome. Nine children with INS (aged 27–15.7 years) were studied. Controls consisted of 9 healthy children (aged 1.9–15.3 years). Lymphocytes were isolated from heparinized blood via density gradient centrifugation, washed and suspended in phosphate buffered saline supplemented with 15% heat inactivated fetal calf serum. Aliquots of 100 μl were incubated with PHA (15 μg/ml) and prednisolone (concentrations 0–280 μmol) then labeled with tritiated thymidine. Thymidine incorporation into DNA was measured by liquid scintillation counting. Suppression of lymphocyte blastogenesis was expressed as the inhibitory dose 50% (ID_{50}) of prednisolone calculated from the prednisolone dose response curve. Lymphocytes from nephrotic patients showed in vitro resistance to prednisolone suppression with ID_{50} 77.8 ± 18.5 (mean ± SE) compared with healthy controls ID_{50} 6.8 ± 3.2 ($P = 0.0037$). This study further implicates lymphocyte function in the mechanisms underlying INS. However, the study did not reliably predict clinical response of INS to prednisolone.

Effect of dietary NaCl on intramyocardial and renal vascular and glomerular lesions in hypertension. *D.T. Liu, P. Kincaid-Smith, and J.A. Whitworth, Department of Medicine, St. George Hospital, University of New South Wales, and Department of Nephrology, Royal Melbourne Hospital, Australia.* To examine the hypothesis that high levels of dietary NaCl aggravate hypertension-associated vascular lesions, a study was carried out in DOCA, two kidney one clip (2K1C) hypertensive and normotensive control Sprague-Dawley rats. Animals in each group ($N = 8$–10) received regular, high or low NaCl diet (NaD) for 8 weeks. SBP was measured via tail cuff and metabolic balance was measured in metabolic cages weekly. At the end of the study, hearts and kidneys were harvested for histologic study. Diet did not affect SBP in control rats. High NaD significantly increased SBP in DOCA rats, but not in 2K1C rats.

	Na Diet	Control	DOCA	2K1C
SBP	Regular	95 ± 2	118 ± 4	178 ± 9
mm Hg	High	99 ± 3	143 ± 5[a]	172 ± 7
at week 8	Low	103 ± 3	113 ± 5	173 ± 7

[a] $P < 0.01$ vs. regular and low Na diet

Urinary Na excretion was significantly elevated by Na loading in control, DOCA and 2K1C rats ($P < 0.01$). Histologic studies showed high NaD caused a lower ratio of lumen to outer diameter of intramyocardial arteries in DOCA (high NaD 0.33 ± 0.02 vs. regular 0.39 ± 0.02 and low NaD 0.41 ± 0.02) and 2K1C rats (high NaD 0.30 ± 0.02 vs. regular 0.39 ± 0.02 and low NaD 0.39 ± 0.02, $P < 0.005$). It also caused a higher percentage of glomerular lesions in DOCA (high NaD 14% range 1–36% vs. regular 1% range 0–3% and low NaD 1% range 0–4%, $P < 0.05$) and 2K1C rats (high 21% range 1–43% and regular NaD 22% range 0–44% vs. low NaD 5% range 0–40%, $P < 0.05$). For interlobular arterial lesions, high NaD resulted in a higher score in 2K1C rats (high NaD 307 range 200–314 vs. low NaD 200 range 150–250, $P < 0.05$). In conclusion, high NaCl diet caused more severe vascular and glomerular damage in both salt-dependent and salt-independent hypertension, although it has no effect on normotensive animals. This effect was independent of blood pressure.

Analysis of pH recovery after exercise in human and rat hypertension suggests increased proton efflux. *G.J. Kemp, C.H. Thompson, D.J. Taylor, G.K. Radda, and J.G.G. Ledingham, MRC Biochemical and Clinical Magnetic Resonance Unit, John Radcliffe Hospital, Oxford, United Kingdom.* Increased Na/H exchange in vascular smooth muscle and other cells may be important in hypertension. In the spontaneously hypertensive rat (SHR) and human hypertension, exercising skeletal muscle acidifies less than controls, and in SHR this difference is abolished by amiloride. In skeletal myoblasts from SHR, Na/H antiporter activity is increased, but its reported characteristics in vitro differ widely and little is known about its activity in vivo. We present a novel analysis of pH recovery after exercise which, by calculating proton production due to phosphocreatine (P_{Cr}) resynthesis and the titration of cellular buffers, allows quantification of apparent proton efflux. Leg muscles of anesthetised SHR and Wistar-Kyoto (WKY) rats ($N = 5$ each) were studied by ^{31}P magnetic resonance spectroscopy in a 7 T magnet for 15 minutes after sciatic nerve stimulation (10 Hz for 2 min), and spectra used to calculate [P_{Cr}] and pH. In SHR, the early (0–2 min) rate of pH recovery was greater than in WKY (0.21 vs. 0.11 pH unit min^{-1}, respectively, $P < 0.01$). At the start of recovery proton efflux was greater in SHR (17 vs. 10 mmol kg wet wt^{-1} min^{-1}, $P < 0.01$) despite the higher pH (6.30 vs. 6.17, $P < 0.01$). During recovery, proton efflux was negatively related to intracellular pH, with a slope which was increased in SHR (−26 vs. −11 mmol kg · wet wt^{-1} · min^{-1} pH $unit^{-1}$, $P < 0.01$). In humans, similar analysis of recovery from forearm exercise suggests that initial proton efflux is increased in hypertensive patients ($N = 9$) compared to controls ($N = 12$; 13 vs 8 mmol l^{-1} min^{-1}), despite a higher pH (6.55 vs. 6.40).

Proteinuria and its assessment in normal and hypertensive pregnancy. *E. Gallery, V. Kuo and G. Koumantakis, Departments of Renal Medicine, Biochemistry and Obstetrics, Royal North Shore Hospital, St. Leonards, NSW, Australia.* Pregnancy-associated hypertension (P-AH) is a common complication of pregnancy. Proteinuria is a hallmark of its severity, but methods of reporting it are poorly described and standardized, the most commonly recorded method being dipstick analysis of a random urine sample. The purposes of this study were: (1) to determine 24 hour urinary protein excretion rates in normal human pregnancy; (2) to assess the reliability of assessment of proteinuria by dipstick measurement. At 17 to 20, and at 33 to 36 weeks of pregnancy, 174 normal women collected a 24 hour urine sample, whose volume, protein and creatinine concentrations were measured. The result for protein was compared with dipstick analysis of an early morning mid-stream urine sample collected at the end of the 24 hour period. Sixty-eight consecutive inpatients admitted to the antenatal ward with

hypertension and positive urine dipstick tests for protein underwent the same procedure. The inter-observer variability for 66 hospital staff members in dipstick analyses of urine samples of known protein content was assessed. The upper 95% confidence limit of normal was below 0.2 g/24 hours, at both stages of pregnancy investigated. A high proportion of false positive and false negative results was found with dipstick analyses. Assessment of proteinuria by dipstick gave an 18% false positive rate, and a 40% false negative rate for samples with 0.03 g albumin/100 ml urine. Even in the presence of 0.1 g/100 ml, the false negative rate was 7%, while the concentration of protein was significantly underestimated in some 20% of samples with 0.5 g/100 ml. Dipstick urinalysis cannot be relied upon either to detect or to exclude the presence of proteinuria in pregnant women.

Metabolic effects of angiotensin converting enzyme inhibitors (ACE'Is) in healthy volunteers. *M.O. Pratt, N.J. Lewis-Barned, and R.J. Walker, Department of Medicine, Otago Medical School, Dunedin, New Zealand.* Insulin resistance is a etiological factor in non-insulin-dependent diabetes mellitus, hypertension, obesity and accelerated atherosclerosis. Captopril may improve insulin sensitivity in hypertensive individuals. No studies have compared the effect of different ACEI's and their effect on insulin sensitivity in a normal population. Ten healthy volunteers (8 males, 2 females) were enrolled in a randomized, single-blind cross over study comparing captopril (6.25 mg twice a day for 28 days) with enalapril (5 mg per day for 28 days) with a 28-day wash out period between drugs. All 10 subjects completed the trial with no change in their dietary habits or exercise level during the study. Insulin sensitivity (M value mg/kg/min) was measured using the euglycemic hyperinsulinemic clamp at the start and completion of each four week drug treatment. Blood pressure, plasma biochemistry, glucose and insulin were monitored throughout the study. The insulin sensitivity, insulin sensitivity index (ISI), fasting insulins and plasma glucose showed no first or second order carryover effects.

		Day 0	Day 28
M value	Captopril	6.3 ± 0.6	5.6 ± 0.7 mg/kg/min
	Enalapril	6.1 ± 0.4	5.4 ± 0.5 mg/kg/min
ISI	Captopril	8.7 ± 1.3	7.4 ± 1.1 M/μU/ml%
	Enalapril	8.0 ± 0.8	7.4 ± 1.0 M/μU/ml%
Insulin	Captopril	7.9 ± 0.9	11.5 ± 2.2 μU/ml
	Enalapril	9.7 ± 1.5	11.5 ± 1.4 μU/ml
Glucose	Captopril	4.3 ± 0.1	4.1 ± 0.1 mmol/liter
	Enalapril	4.3 ± 0.1	4.2 ± 0.1 mmol/liter

The decreased M value and ISI demonstrated just failed to reach significance (95% confidence range $P < 0.056$). The fasting insulin increased significantly ($P < 0.05$), and the fasting glucose decreased but failed to reach significance. There was no significant difference demonstrated between captopril and enalapril. Of considerable interest is a decreased ISI which is opposite to the trend seen in hypertensive individuals. Explanation for this can't be attributed to factors known to modulate insulin sensitivity or to the actions of captopril alone.

Urinary red blood cell and cast excretion in normal and hypertensive human pregnancy. *E.D.M. Gallery, M. Ross, and A.Z. Gyory, Department of Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia.* Although the well-recognized pregnancy changes in renal blood flow, cellular function, tubular fluid composition and flow rates may cause altered excretion rates of formed elements in the urine, relatively little attention has been paid to this in normal pregnancy. The urinary white cell excretion is known to be increased, with loss of the usual relationship between pyuria and urinary infection. Whether the same is true for other formed elements is unknown. This study's purpose was to determine normal values for excretion of erythrocytes and casts, and to establish whether they became abnormal in women who developed pregnancy-associated hypertension (P-AH). Study subjects were 174 continuously normotensive pregnant volunteers (N), 22 who developed P-AH, and 8 with chronic essential hypertension (CEH). Early morning mid-stream urine samples were collected at 17 to 20 and at 33 to 36 weeks' amenorrhea, after insertion of a vaginal tampon. Urinary microscopy was performed by standard techniques (Kesson et al: *Lancet* 2:809, 1978). The 95th percentile for concentration of erythrocytes was ≤2500/ml and for casts ≤30/ml in normal pregnancy. Values for those who developed P-AH and for the small group with CEH were within these limits.

ACTH hypertension in the rat: Role of sodium chloride. *Ming Li and Judith A. Whitworth, Department of Medicine, St. George Hospital, University of New South Wales, Australia.* Adrenocorticotrophin (ACTH) produces adrenally dependent increases in both blood pressure and salt (NaCl) appetite in the rat. ACTH treatment induced a large increase in blood pressure in the intact rat on normal and low sodium diet. The present study examines the effect of free access to a high intake of NaCl on ACTH hypertension in the rat to test the hypothesis that high NaCl intake would amplify the rise in blood pressure. Either water or 1% NaCl were offered to sham or ACTH treated Sprague-Dawley rats. Subcutaneous injections of synthetic ACTH (0.5 mg/kg/day) for 10 days caused large increases in the intake of both 1% NaCl (+240 ± 6 ml/day) and water (+45 ± 4 ml/day), urine volume (1% NaCl + ACTH +182 ± 4 ml/day, ACTH + water + 36 ± 2 ml/day), adrenal weight (ACTH + water 176 ± 18, ACTH + 1% NaCl 367 ± 129 mg/100 g body wt) and maximum systolic blood pressure (SBP) (ACTH + water +18 ± 5 mm Hg; 1% NaCl + ACTH +16 ± 3 mm Hg). The rise in systolic blood pressure following ACTH in water drinking rats was higher than that in animals on 1% NaCl at treatment day 10 (ACTH + water 116 ± 5; 1% NaCl + ACTH 104 ± 6 mm Hg; $P < 0.05$). This difference may reflect the thoracicoabdominal edema seen in 1% NaCl drinking rats on ACTH. Three rats developed very low blood pressure (55, 75 and 90 mm Hg, respectively) by treatment day 8 through 10, suggesting compromised cardiac function. ACTH treatment in rats offered 1% NaCl produced significant increases of urinary sodium and potassium excretion probably reflecting the huge increase of urine volume compared to ACTH treatment in water drinking rats, or sham injected rats on 1% NaCl. Plasma potassium in ACTH treated 1% NaCl drinking rats was significantly lower than in ACTH treated water drinking rats (ACTH + water 3.7 ± 0.2 mmol/liter ACTH + 1% NaCl 2.3 ± 0.4 mmol/liter, $P < 0.001$), but there was no difference in plasma sodium concentration. Thus, free access to NaCl in ACTH treated Sprague-Dawley rats did not potentiate the hypertension, but was associated with edema in some animals.

Clinicopathological course of microscopic polyarteritis (MPA). *E.A. D'Almeida, R.S. Nanra, S.C. Carney, A.H.B. Gillies, B.F. Jones, and P.R. Trevillian, Nephrology Unit, John Hunter Hospital, Newcastle, NSW, Australia.* Data from 27 male and 20 female patients, age 51 ± 19 years, with MPA, and followed for 3 to 180 months (median 26), were reviewed retrospectively. The diagnosis of MPA was made on the renal biopsy finding of a focal proliferative glomerulonephritis with no immune deposits detected by immunofluorescence. Seventy percent of biopsies ($N = 40$) had cellular crescents, and 18% had crescents in >50% of glomeruli. Vasculitic lesions were absent in all biopsies. Histological activity grade on a scale of 0 to 3 was: 0 = 2%, 1 = 37%, 2 = 41% and 3 = 20%. Chronicity grade on a scale of 0–2 was: 0 = 7%, 1 = 81% and 2 = 12%. The major nonrenal manifestations were: fever 52%, musculoskeletal 39%, weight loss 38%, vasculitic rash 22%; other organ involvement occurred in 2 to 7% of patients. Renal manifestations were: proteinuria 100%, hematuria 96%, BP > 150/90 mm Hg 76%, S_{Cr} > 0.15 55% (range 0.06–1.17, median 0.17), RBC casts 53%, nephrotic syndrome 32%, and WBC casts 17%. Serum IgG was elevated in 36%. Data on treatment were available in 35 patients and consisted of: low-dose prednisone (<40 mg/day) in 5 patients, and high-dose prednisone (>40 mg/day) in 30 patients with additional cyclophosphamide in 19 patients. Four patients died, 2 commenced maintenance dialysis, and 4 were lost to follow-up. Of the remaining 37 patients, renal function improved in 37% (median S_{Cr} 0.39–>0.15), remained stable in 33% (median S_{Cr} 0.11–>0.11), and deteriorated in 30% (median S_{Cr} 0.19–

>0.37). The predictors of renal deterioration were: elevated serum IgG ($P = 0.005$), % of glomeruli with active focal lesions ($P = 0.026$), low dose prednisone ($P = 0.057$), histological activity of disease ($P = 0.067$), and % of glomeruli with global glomerular sclerosis ($P = 0.070$). (mean ± standard deviation; S_{Cr} = serum creatinine mmol/liter)

Renal functional abnormalities in association with rheumatoid arthritis (RA). *P. Savvas, L.S. Ibels, A.Z. Gyory, and P.M. Brooks, Departments of Renal Medicine and Rheumatology, Royal North Shore Hospital, St. Leonards, NSW, Australia.* A prospective study was undertaken to determine abnormalities in patients with RA, with special reference to tubular function. Forty-six patients were assessed by clinical examination, serology, 24-hour urinary protein excretion and comprehensive one-day renal function testing. All patients fulfilled revised ARA criteria for RA. RA had been present for 2 months to 49 years (mean 11 years) at the time of study. True serum creatinine concentration was abnormal in only 1 patient. Abnormal proteinuria was detected in 10. Creatinine clearance, when adjusted for age and sex, as abnormal in 5. Proximal tubule function, assessed by 15 minute phenol-sulphonphthalein excretion, was abnormal in 15. White cell excretion rate was elevated in 26. Centrifuged urine microscopy revealed abnormal cast excretion in 35 (waxy in 35, granular in 25, hyaline in 14). Pyuria and microscopic hematuria were also frequent (24 and 12 patients, respectively). Maximum urinary osmolarity after intranasal antidiuretic hormone was abnormal in 31, and 43 patients had a defect in urinary acidification after ammonium chloride load, indicative of tubular dysfunction. Only 2 patients had entirely normal renal function. Correlations will be presented with clinical and immunological parameters as well as drug intake, especially non-steroidal anti-inflammatories. Thus, patients with long-standing RA have a very high incidence of renal functional abnormalities despite normal serum creatinine concentrations. These predominantly reflect medullary or tubulointerstitial disease. This should be acknowledged when prescribing potentially nephrotoxic drugs in this group. Elevated serum creatinine concentration alone is the most inaccurate way of assessing renal function in RA patients.

Calcium-related changes with evolving renal failure. *M. Cochran, H. Morris, Department of Medicine, Flinders Medical Centre, Bedford Park, Institute of Medical and Veterinary Science, Adelaide, Australia.* The origin of renal secondary hyperparathyroidism is now becoming clearer. Recent studies suggest that the primary defect lies with a failure of adequate production of 1,25D at a relatively early stage of renal insufficiency. In a cross-sectional study we have measured calcium-related variables in 180 subjects whose collective range of renal function spanned normality to severe impairment. The renal function was categorized from a derived creatinine clearance using age, weight, and plasma creatinine. The earliest change, at creatinine clearance 70 ml/min, was a fall in fasting and non-fasting urine calcium excretion, though the excretion relative to GFR was unaltered. Plasma calcium and phosphate were unchanged. The serum 1,25D was not detectably lower at this stage, but PTH showed a small rise. As renal function declined to a creatinine clearance of 30 ml/min, the plasma calcium began to fall and absolute urine calcium excretion reached a minimum. Plasma phosphate decreased slightly and calcium absorption and 1,25D were clearly decreased. The PTH was mildly elevated. At lower levels of creatinine clearance, the phosphate increased with the plasma creatinine, the 1,25D level decreased further and PTH was more elevated. Plasma calcium was reduced but urine calcium increased. The calcium-related changes at different levels of renal failure are complex, but the data suggest that phosphate loading is not generally the initiating factor in decreased production of 1,25D. They also lend support to the notion of decreased tissue sensitivity to the metabolite.

Effect of recombinant human growth hormone (rhGH) on calcium regulation in short, slowly growing (SSG) children. *G.D. Ogle, A.R. Rosenberg, and G. Kainer, Department of Nephrology, Prince of Wales Children's Hospital, Randwick NSW, Australia.* Growth hormone (GH) affects calcium homeostasis, but the effects of GH therapy on calcium regulation in non GH-deficient SSG children are unknown. We prospectively studied 11 prepubertal SSG children (8 boys and 3 girls) with normal 24-hour GH secretion. Age range was 4.9 to 12 years (mean 9.6 yr). rhGH was administered subcutaneously in a mean dose of 0.55 U/kg/wk. The results (expressed as means ± SD) were as follows:

	Baseline	8 weeks	24 weeks
Serum calcium *mmol/liter*	2.40 ± 0.09	2.40 ± 0.09	2.38 ± 0.09
Serum phosphate *mmol/liter*	1.52 ± 0.12	1.62 ± 0.24	1.6 ± 0.22
Urine calcium *mmol/kg/24 hr*	0.063 ± 0.039	0.049 ± 0.049	0.054 ± 0.056
Urine phosphate *mmol/24 hr*	13.6 ± 5.0	17.4 ± 10.3	19.0 ± 8.3[a] $N = 10$
Serum 1,25 $(OH)_2$ vitamin D_3 *pmol/liter*	71.2 ± 38.5	111.8 ± 32.0[a] $N = 9$	122.9 ± 49.4[b] $N = 9$
Serum calcitonin *pg/ml*	85.2 ± 31.6	77.4 ± 39.3	74.0 ± 16.5
Serum parathyroid hormone *pmol/liter*	1.96 ± 0.94	1.81 ± 0.71	2.05 ± 0.96

[a] $P < 0.05$, [b] $P < 0.02$, compared to baseline, by Student's t-test

We have shown for the first time in non GH-deficient children that rhGH increases 1,25 dihydroxyvitamin D_3. This prolonged effect was not associated with significant changes in other calcium regulating hormones (however, surprisingly, urinary phosphate increased), and may have adverse clinical implications.

Comparison of cyprofloxacin with netilmicin for treatment of acute pyelonephritis: Reducing hospital stay. *R.R. Bailey, K.L. Lynn, R.A. Robson, B.A. Peddie, and A.H. Smith, Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.* The aminoglycoside antibiotics are still considered the drugs of choice for the treatment of severe or complicated urinary tract infections. The 4-quinolones are challenging the aminoglycosides regarding efficacy and safety. A prospective randomized study was undertaken to compare the efficacy of cyprofloxacin and netilmicin for the treatment of patients with acute pyelonephritis requiring hospitalization. Forty-three patients were enrolled in the study and 34 (29 women) completed the protocol. Fifteen of 17 patients treated with cyprofloxacin (100 mg 12 hr. i.v., switching when clinically appropriate to 250 mg 12 hr. orally) were cured. Fifteen of 17 treated with netilmicin (2 mg/kg loading dose i.v. and then adjusted according to drug concentrations and renal function) were cured. All patients were treated for 5 days. One patient relapsed after cyprofloxacin and another had a re-infection, while 2 relapsed after netilmicin. Five of the 6 patients with a urinary tract abnormality were cured. Side effects were generally mild and rapidly reversible. Patients treated with cyprofloxacin spent a mean of 3.7 days in the hospital compared with 5.3 days for those treated with netilmicin. The difference in duration of hospital stay was statistically significant ($P < 0.01$). In summary, both cyprofloxacin and netilmicin proved highly effective and safe for the treatment of severe acute pyelonephritis.

Hemodialysis increases serum alpha-2 macroglobulin level independently of the membrane used. *A. Argilés, P.G. Kerr, C. Mion, and R.C. Atkins, Departments of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia, and Lapeyronie University Hospital, Montpellier, France.* Beta-$_2$ microglobulin (β_2m) has been documented as the major protein constituent of hemodialysis associated amyloidosis (HDAA). However, the pathogenesis of HDAA is still unresolved and other proteins have been implicated. We previously identified alpha-$_2$ macroglobulin (A_2M) in amyloid deposits and suggested that A_2m may participate in the formation and/or maintenance of the deposits. In the present study we analyzed the influence of the hemodialysis (HD) procedure and membranes on A_2m serum levels. Seven stable dialyzed patients (3 F, 4 M; 60 ± 2 year old) participated in the study. A_2m,

alpha-1 antitrypsin (both measured by nephelometry), β_2m (by RIA) and total proteins were monitored hourly during HD and 2 and 8 hours after HD during 9 consecutive dialyses (3 cycles of 3 HD each, alternatively utilizing AN69 or cuprophane membranes in a cross-over design). All dialyses were 4 hours with bicarbonate dialysate. A_2m significantly increased from hour 3 and peaked 2 hours post-HD (+11% and +9% with AN69 and cuprophane, respectively; $P < 0.001$ by paired t-test). Total proteins peaked at hour 4 (+4% and +3%; $P < 0.01$) and decreased after HD. β_2m significantly decreased during AN69 HD (−29%; $P < 0.001$) and remained unchanged during cuprophane HD (+3%; P = NS). In conclusion, HD induces an increases in serum levels of A_2m regardless of the membrane used. We postulate that these findings are of relevance for HDAA pathogenesis since A_2m, previously identified in HDAA deposits, is closely related to the acute phase reaction proteins and could represent a new manifestation of an inflammatory response to the dialysis procedure.

Comparison of intermittent intravenous (i.v.) versus oral (PO) calcitriol for the treatment of secondary hyperparathyroidism in hemodialysis (HD) patients. *E.R. Fischer and D.C.H. Harris, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* Both intermittent i.v. and intermittent PO calcitriol have been shown to be effective in the treatment of secondary hyperparathyroidism in HD patients, and it has been claimed that i.v. calcitriol causes less hypercalcemia. However, there has been no systematic comparison of the two routes of administration. Therefore, in a crossover study, 11 (9 male) patients (age 45.5 ± 5.5 SEM) on maintenance HD were randomized to receive i.v. followed by PO calcitriol for 4 months each, or PO followed i.v. calcitriol, commencing at 2 μg post-dialysis. Calcium-containing phosphate binders were not used. Calcitriol was ceased if hypercalcemia developed and restarted at 2 or 1 μg when calcium returned to normal. Hypercalcemia was frequent—11 episodes in 8 patients on i.v. calcitriol, and 10 in 7 patients in PO. Dose reduction to 1 μg was necessary in 6 patients on i.v. and the same 6 patients on PO. Serum immunoreactive parathyroid hormone (normal <65 pg/ml) fell similarly after 12 weeks of i.v. (406 ± 119 to 301 ± 89 pg/ml) or PO (306 ± 57 to 240 ± 57, $P = 0.04$) calcitriol. Parathyroid enlargement was seen in 4 patients on ultrasound; no size reduction was demonstrated after the first 4 months. Similarly, there was no reduction in activity on quantitative metabolic bone scan in either group after 4 months. In summary, intermittent oral calcitriol and intermittent intravenous calcitriol are equally effective in reducing serum parathyroid hormone, and at a dose of 2 μg postdialysis cause hypercalcemia with equal frequency.

Echocardiographic assessment of cardiac effects of erythropoietin in hemodialysis patients. *K.L. Lynn, A.L. Buttimore, J.A. Inkster, D. Divakar, A.L. Mylius, H. Ikram, B.I. Shand, R.R. Bailey, R. Robson, and J.E. Wells, Departments of Nephrology and Cardiology, Christchurch Hospital and Christchurch School of Medicine, Christchurch, New Zealand.* The effects of erythropoietin (EPO) on cardiac function were studied over 12 weeks. Twenty home hemodialysis patients were randomly allocated to either EPO 50 U/kg or placebo i.v. three times weekly. No patients were on antihypertensive drugs. 2-D guided M-mode echocardiograms were performed at baseline and 12 weeks. Hemoglobin ($P < 0.001$) and high shear rate blood viscosity ($P = 0.006$) increased on EPO. Blood pressure did not change. Sixteen (8 EPO) patients had complete echocardiographic data.

Variable[b]	Adjusted means[a] diff Placebo	EPO	(EP)	P ANCOVA
Ejection fraction %	64.8	75.4	10.6	0.05
Index fract short %	36.2	45.3	9.1	0.05
Heart rate *b/min*	66.5	61.9	−4.6	0.07
Myocardial thick *mm*	14.2	12.2	−2.0	0.15
LV mass *g*	358	275	−83	0.24
L atrial diam *mm*	36.2	32.4	−3.8	0.15

[a] Prestudy value used as covariate
[b] LV dimensions did not change significantly

EPO therapy for 12 weeks resulted in improvement in myocardial function and an encouraging, but non-significant, reduction in cardiac mass.

Long-term effect of low calcium dialysate on parathyroid activity in dialysis patients treated with calcium carbonate as a phosphate binder. *W.E. James, C. Doecke, G. Allen, M. Cochran, and L. Barratt, Department of Medicine, Renal Unit, Flinders Medical Centre, SA, Australia.* It has been suggested that long-term low calcium dialysate bath (L.C.D.B.) use may contribute to renal hyperparathyroidism. We reviewed the results of 49 hemodialysis patients at Flinders Medical Centre (F.M.C.) between November 1988 and July 1991 to assess whether L.C.D.B. was provoking hyperparathyroidism. Twenty-nine patients were excluded due to complicating illness or alteration of their regimen. Of the remaining 20 patients, 13 received a L.C.D.B. (defined as a calcium dialysate bath ≤1.2 mmol/liter) and 7 patients received a normal calcium dialysate bath (N.C.D.B., bath ≥1.4 mmol/liter). The percentage change of parathyroid hormone over the period of review was +4.9% in the L.C.D.B. group and +0.4% in the N.C.D.B. group; these results were not significantly different. There was a significant rise ($P < 0.05$) in the serum alkaline phosphatase level in both groups during the period of review, L.C.D.B. +63%, and N.C.D.B. +37%. There was a significant difference between the mean predialysis phosphate in the L.C.D.B. group (1.84 mmol/liter) and the N.C.D.B. group (1.55 mmol/liter), $P < 0.01$. Predialysis hypercalcemia (corrected calcium ≥2.75 mmol/liter) occurred in 12.6% of the L.C.D.B. group, which was significantly different to the N.C.D.B. group (5.9%), $P < 0.005$. A L.C.D.B. and the use of calcium carbonate as a phosphate binder does not worsen existing parathyroid activity, is associated with acceptable control of interdialytic hyperphosphatemia, and increases the risk of predialysis hypercalcemia. Both groups were associated with a significant rise in sALP over the period of review despite stable parathyroid over-activity.

Effect of essential fatty acid deficiency (EFAD) on renal allograft and xenograft survival. *A.J.F. d'Apice, S. Kalina, A. McKenzie, M. Polihronis, P. Eadie, B. Pollock, A.J. Sinclair, M.J. Pearse, and B.McC. O'Brien, Departments of Nephrology and Clinical Immunology and Microsurgery Research Centre, St. Vincent's Hospital, Fitzroy, Australia.* In 1988, Schreiner et al (*Science* 240:1032) reported that kidneys from EFAD Lewis rats were profoundly depleted of glomerular and interstitial cells which bore Ia and macrophage antigens. When transplanted to untreated BN rats, these kidneys survived indefinitely. This observation was widely interpreted as confirmation of the importance of passenger leukocytes or dendritic cells in the induction of the allogeneic response and potentially of clinical importance in prevention of xenograft rejection. This study was undertaken to confirm the primary observation. Lewis rats and Balb/c mice were weaned at 21 days and placed on either an EFAD diet (EFAD#1) or a normal diet. A further group of Lewis rats was placed on a more stringent EFAD diet (EFAD#2). Donors were aged between 2 and 3 months at the time of transplant. Recipient DA rats were fed normal diets and were aged 2 to 3 months at transplantation. The donors' left kidney was used for transplantation and the right kidney and liver were used for lipid analysis and immunohistology. Interstitial macrophages were enumerated using anti-Ia monoclonal antibodies by the peroxidase anti-preoxidase technique. Lipid analysis was performed on chloroform-methanol extracted tissues by capillary gas liquid chromatography.

Group (Donor/diet/recip)	N	IA (+) cells /10 HPF	$\omega 9/\omega 6$ ratio	Graft survival (mean ± SD)
Le/EFAD#1 → Le	5	ND	2.2 ± 0.4	>90 days
Le/control → DA	9	13.5 ± 2.9	0.06 ± 0.06	8.4 ± 2.4
Le/EFAD#1 → DA	7	ND	1.7 ± 0.6	6.3 ± 1.1
Le/EFAD#2 → DA	7	22.8 ± 3.6	2.4 ± 1.2	5.9 ± 1.2

Induction of marked EFAD, did not reduce interstitial Ia (+) cells and there was no prolongation of graft survival of EFAD donor kidneys. Similar results were obtained with mouse to rat renal xenografts.

Glomerular (GP) and tubular proteinuria (TP) as markers of renal deterioration in analgesic (AN) and reflux nephropathy (RN). *R.S. Nanra, Nephrology Unit, John Hunter Hospital, Newcastle, NSW,*

Australia. To evaluate the effect of GP and TP on renal function, urine albumin excretion ($U_{Alb}V$ mg/mmol Cr) and β_2-microglobulin excretion ($U_{\beta_2m}V$ μg/mmol Cr) were measured in 52 AN (age 61 ± 9 years, C_{Cr} 4–64, median 38) and 25 RN (age 40 ± 15 years, C_{Cr} 10–88, median 55) patients. In AN patients, total proteinuria (N = 40) was 0.03–4.39 g/day (median 2.2), $U_{Alb}V$ (N = 50) was 1–1,738 (median 652), and $U_{\beta_2m}V$ (N = 52) was 13–23,773 (median 9,712); the correlation coefficient "r" for C_{Cr} vs. $U_{Alb}V$ was 0, and for C_{Cr} vs. $U_{\beta_2m}V$ was −0.53 (P = 0.0005). In RN patients, total proteinuria (N = 22) was 0.02–9.44 g/day (median 4.11), $U_{Alb}V$ (N = 25) was 1–210 (median 110), and $U_{\beta_2m}V$ (N = 25) was 15–6,482 (median 2,749); "r" for C_{Cr} vs. $U_{Alb}V$ was −0.53 (P = 0.006), and for C_{Cr} vs. $U_{\beta_2m}V$ was −0.52 (P = 0.008). Of 37 AN patients followed for 12–192 months, 20 had stable and 17 had declining C_{Cr}. $U_{Alb}V$ in the stable group (1–1,738, median 30) and deteriorating group (2–402, median 65) were similar (P = 0.15), but $U_{\beta_2m}V$ in the stable group (23–10,217, median 131) was less than in the deteriorating group (range 44–8,835, median 3,339) (P = 0.081). Of 25 RN patients followed for 12–168 months, 16 had stable and 9 had declining C_{Cr}. $U_{Alb}V$ in the stable group (1–208, median 38) and deteriorating group (4–210, median 68) were similar (P = 0.18), but $U_{\beta_2m}V$ in the stable group (15–4,800, median 36) was less than in the deteriorating group (63–6482, median 484) (P = 0.053). These data suggest that in AN and RN, increase in TP but not GP is a predictor of renal deterioration. C_{Cr} = creatinine clearance ml/min/1.73 m^2 surface area, data are Mean ± standard deviation.

Risk factors for renal and pelvic cancer. *Margaret McCredie, and J.H. Stewart, Cancer Epidemiology Unit, NSW Cancer Council; and Renal Medicine, Westmead Hospital, NSW, Australia.* A population-based case-control study of renal cell carcinoma (RCC) and renal pelvic carcinoma (TCC) has been undertaken in NSW, 10 years after compound analgesics were banned from sale over-the-counter. A standard questionnaire seeking information on medical history and consumption of prescribed drugs, analgesics and tobacco was used in interviews with cases of RCC (N = 503) and TCC (N = 149) diagnosed in 1989–90 and 535 controls from a proportional random sample of the electoral rolls. Analgesic use was evaluated in four mutually exclusive categories—aspirin and paracetamol (each as single drugs), aspirin/phenacetin compounds and aspirin/paracetamol compounds. Risk ratios (RR) were estimated by multivariate logistic regression. Significant risk factors and their RR (each adjusted for the effect of all other significant factors) were:

	RCC	TCC
Ex-smokers	1.5	1.2
Current smokers	2.3	4.3
Body mass index—highest tertile	1.7	—
Diet pills	1.8	—
Hypertension before 1970	1.9	—
Beta-blockers	1.5	2.1
Kidney injury	4.0	3.8
Kidney infection	—	1.8
Lower urinary infection	0.6	—
Phenacetin compounds	—	8.1

An increasing risk with increasing use was found for phenacetin-containing compounds (TCC only) and smoking (RCC, TCC). No significantly increased risk was found for kidney stones, aspirin/paracetamol compounds, aspirin or paracetamol.

The pattern of renal reserve (RR) in renal diseases. *R.S. Nanra, S.K. Nanra, and A.H.B. Gillies, Nephrology Unit, John Hunter Hospital, Newcastle, NSW, Australia.* To evaluate the pattern of RR in renal diseases, a pilot study was performed in 61 subjects (aged 13–72 years, median 31) with glomerulonephritis (N = 24), analgesic (AN), reflux nephropathy (RN) (N = 23), polycystic kidney disease (N = 9), hypertension (N = 3), and normal (N = 2). RR was measured by the increase in hydrated C_{Cr} during infusion of a low-sodium amino acid solution. The other indices which were measured were: 24-hour urea excretion ($U_{Urea}V$ mmol/mmol Cr) on the day before the RR test, the mean arterial pressure (MAP mmHg), basal and increase in albumin excretion (B-$U_{Alb}V$ and ˆ $U_{Alb}V$ mg/mmol Cr), and basal and increase in β2Microglobulin excretion (B-$U_{\beta_2m}V$) and ˆ U_{β_2m} μg/mmol Cr). The basal C_{Cr} was 11 to 147 (median 83.5) and the range of RR was −8 to +36 (median 4.5). Thirty-nine subjects (mean age 39 ± 16 years) had no increase in C_{Cr} (RR −8 to +8), (RR(−), and 22 (mean age 33 ± 15 years) had an increase in C_{Cr} (RR >9), (RR(+)). The RR(−) group had more AN and RN patients (N = 20 vs N = 3, P = 0.031), a lower basal C_{Cr} (median 75 vs. 102, P = 0.0029), a greater increase in $U_{\beta_2m}V$ (median 100 vs. −100, P = 0.0002), and a higher $U_{Urea}V$ (mean 36.0 ± 13.3 vs. 29.5 ± 9.5, P = 0.051). There were no differences between RR(−) and RR(+) groups in age, B-$U_{Alb}V$ and ˆ $U_{Alb}V$, B-$U_{\beta_2m}V$, and MAP. These data suggest that: (1) there may be differences in RR between glomerular and tubulointerstitial diseases, (2) RR may be influenced by C_{Cr} and protein intake, and (3) RR appears to be associated with changes in proximal tubular handling of β_2m. C_{Cr} = creatinine clearance ml/min/1.73 m^2 surface area, data are mean ± standard deviation.

Nephrocalcinosis in pre-term babies. *P. Sivakumar, M. Murnane, C. Rodda, V. Yu, and M. McIver, Neonatal Intensive Care and Paediatric Renal Unit, Monash Medical Centre, Clayton, Victoria, Australia.* Nephrocalcinosis is being identified with increasing frequency in pre-term infants. The etiology, generally considered to be due to frusemide, is not clear. Neither is the incidence or the long-term consequences in babies who survive. A preliminary survey of all pre-term infants of less than 29 weeks gestation admitted to Monash Medical Centre over a 10 month period identified 14 infants in the 38 babies who survived more than 2 weeks, in whom an ultrasound diagnosis of nephrocalcinosis was made. The abnormality persisted in 11. All of these babies had chronic lung disease and the diagnosis of nephrocalcinosis was made at a mean age of 35 days. Babies received diuretics, chlorothiazide and spironolactone with or without dexamethasone, together with i.v. fluids containing calcium supplements. No correlation between treatment regimes and nephrocalcinosis could be made. The only significant biochemical difference in babies with persistent nephrocalcinosis was a low serum phosphate. Hypertension was noted in 6 of the babies and persists in 2 who have been followed for more than 18 months. Further study of the pathogenesis of nephrocalcinosis is required. The abnormality is not necessarily benign and these babies need long-term supervision of B.P., renal function, and phosphate metabolism.

Lupus nephritis in pregnancy. *D.K. Packham, S.S. Lam, K. Nicholls, K.F. Fairley, and P. Kincaid-Smith, Department of Nephrology, Royal Melbourne Hospital, Victoria, Australia.* Sixty-four pregnancies undertaken by forty-one women with biopsy-proven lupus nephritis between 1965 and 1991 were analyzed. Of the sixty-five fetuses, 34% were lost (including therapeutic abortions), 30% were live but premature, and 37% were term. Deterioration in maternal renal function was seen in 19% of pregnancies, but in only one woman (2%) was there irreversible post-partum. Hypertension was recorded in 44% of pregnancies; it developed early (<32 weeks gestations) in 28% and was severe (a diastolic of >110 mm Hg) in 13%. Treated hypertension predated 17% of pregnancies, and in 6% exacerbation occurred during pregnancy. Nine women (22%) who developed de novo hypertension in pregnancy had permanent hypertension post-partum. Increased proteinuria was recorded in 48% of pregnancies and was irreversible postpartum in 5%. The presence of the circulating lupus anti-coagulant (LAC) was clearly associated with significantly high fetal loss rate, although the incidence of maternal complications did not differ significantly between LAC-positive and LAC-negative mothers.

Pharmacokinetics of rufloxacin in patients with impaired renal function. *G.J. Perry, T.G.K. Mant, P.J. Morrison, S.H. Sacks, and B.P. Imbimbo, Renal and Drug Research Units, UMDS, Guy's Hospital, London UK and Mediolanum Farmaceutici, Milan, Italy.* Rufloxacin is a new fluoroquinalone characterized by a broad spectrum of activity against gram-negative and gram-positive serobic bacteria. The pharmacokinetics of rufloxacin were investigated in normal subjects and in patients with various degrees of renal failure after the administration of a single oral 400 mg dose. The subjects were classified by glomerular filtration rate (GFR) normalized for body surface area: group 1: GFR of >80 ml/min/1.73 m^2; group 2: GFR of 80 to 31 ml/min/1.73 m^2; group 3:

GFR of 30 to 11 ml/min/1.73 m^2; and group 4: GFR of ≤10 ml/min/1.73 m^2. Each group consisted of 6 subjects. Four out of the six patients in group 4 were on maintenance hemodialysis or peritoneal dialysis. Rufloxacin plasma and urinary concentrations were determined by HPLC and bioassay. A two-compartment model applied on HPLC data was used to calculate rufloxacin pharmacokinetic parameters. Absorption and distribution of rufloxacin were not affected by the renal status of subjects. Clearance of the drug from the body was slow and was influenced by renal function. The elimination half-lives were 28.1 ± 2.5, 33.7 ± 3.2, and 42.4 ± 3.2 in groups 1, 2 and 3, respectively, and did not increase further in group 4 (31.5 ± 5.3 h), probably due to the clearance effect of dialysis. The 0- to 96-hour cumulative urinary recoveries of rufloxacin were 33 ± 3, 27 ± 3, 14 ± 2 of the administered dose in groups 1, 2, 3, 4 ± 2% of the administered dose in groups 1, 2, 3 and 4, respectively. The renal clearances of rufloxacin decreased linearly with the decrease in GFR ($r^2 = 0.848$, $P < 0.001$), while the non-renal clearance did not change significantly between groups. In conclusion, rufloxacin 400 mg/day provides therapeutic plasma concentrations in normals and can be given every 48 hours in patients with moderate to severe renal impairment.

Lupus nephritis in children and adolescents. *A.M. Walker, D.J. Lewis, M. McIver, and N.M. Thomson, Department of Paediatric Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* SLE is uncommon in childhood, but when compared in adults lupus nephritis is thought to be more common and more severe. We have reviewed 33 patients with SLE below the age of 25 years at the time of diagnosis presenting in a 16 year period 1975–91, 28 of whom had evidence of renal involvement. The mean age at presentation was 15.7 years (range 5–24 years). The female to male ratio was 15:1 and included both pre- and post-pubertal patients. All patients filled the A.R.A. criteria for SLE. Mean duration of follow-up was 6.8 years (range 0.1–29 years). Thirteen patients presented with acute disease (serositis 3; nephrotic syndrome 3; fever and severe arthritis 2; vasculitis 2; pulmonary embolus, acute psychosis and thrombocytopenia 1 each) while 5 had sub-acute disease (weeks to 2 months) and 15 had chronic symptoms. Twenty-eight patients (84%) had evidence of SLE nephritis at some stage during their illnesses; they all had proteinuria and/or hematuria, and 3 had abnormal renal function at presentation. Renal biopsy was performed in 26 patients, these all showed lupus glomerulonephritis: WHO Class II, 3; Class III, 2; Class IV (diffuse proliferative), 14; Class V (membranous), 4; and advanced sclerosis 3. Treatment included steroids (31), azathioprine (13), and cyclophosphamide was used in 6 (5 diffuse proliferative and 1 membranous). All patients with Class IIb or III GN have retained normal renal function; however, of the 20 patients with more severe pathology, 3 have serum cr >200 μmol/liter, and 5 have developed ESRF, 3 of whom have died (sepsis).

End-stage renal failure (ESRF) therapy in remote areas for western Australian aborigines: 1988–1991. *M.A.B. Thomas, G. Thatcher, R. Offer, K. Warr, and P. Lee, Renal Unit, Royal Perth Hospital, Perth, WA, Australia.* Australian aborigines from the North-West of WA (1500–2000 km from Perth) have a ≥10 fold incidence of predominantly diabetic and GN end-stage renal failure vs. caucasians; relocation for urban dialysis often produces cultural isolation, depression and non-compliance. Following increasing referrals in 1988, and after consultation with aboriginal groups, local and community health staff with the WA Health Department began a pilot program of (in order of preference) live-related renal transplants, self-care hemodialysis, CAPD or hospital-based IPD (20 liters via multiprong lines over 12 to 18 hours thrice weekly). Monitoring was via local medical officers, with 3-monthly reviews in either Perth or their homes. Of 27 referrals from 1988 to 1991 for ESRF therapy, 4 declined after informed discussion, and 3 relocated to Perth long-term. For 6 LRD Tx, 15 potential donors were excluded by renal disease, medical unfitness, positive cross-match or secondary refusal. Three LRD Tx have died (from non-compliance, infection, amyloidosis), and 3 have stable function (1 recurrent diabetic nephropathy, 1 HTLV1 transmission). 1 cadaveric TX has stable function. Four patients are on self-care HD, with machines located in the home or local hospital for acceptable facilities. Four patients are on CAPD, with peritonitis episodes every 0 to 5 months. IPD in 5 patients produced progressive ultrafiltration failure in 4 (1 now on 5 times weekly IPD, 1 on Perth HD, 2 deaths from withdrawal from treatment), and one rehabilitation transferred to self-care CAPD. Results of all modalities of ESRF management for remote area aboriginals are poorer than for urban caucasian populations, but offer an alternative therapy to patients who would choose to withdraw from treatment rather than relocate for urban dialysis. Survival may be better with dialysis than transplantation in this remote environment.

Pharmacokinetics of intravenously and orally administered lomefloxacin in acute pyelonephritis. *R.A. Robson, R.R. Bailey, K.L. Lynn, N. Hay, G. Pidgeon, Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.* Lomefloxacin is a new, once a day, oral fluoroquinolone with excellent antibacterial efficacy against urinary tract pathogens. An intravenous (i.v.) formulation has been developed recently for the treatment of serious infections. The pharmacokinetics of i.v. and orally (PO) administered lomefloxacin were investigated in patients with acute pyelonephritis. Thirteen patients (12 females) aged 18 to 43 years were enrolled. Each received 400 mg lomefloxacin daily as a single i.v. infusion (30 min) for 3 days and then orally for 4 to 7 days. The pharmacokinetics were evaluated on the last i.v. and first PO day (days 3 and 4). Lomefloxacin was measured in plasma and urine by an HPLC method. Standard methods were used to derive the pharmacokinetic variables (mean ± SD) maximum plasma concentrations (C_{max}), area under the plasma concentration-time curve (AUC_{o-t}), total clearance (CL_p) and absolute bioavailability (F).

	C_{max}	AUC	CL_p	F
i.v.	9.0 ± 2.7	33.4 ± 5.5	204.6 ± 32.5	—
PO	5.5 ± 1.8	32.4 ± 5.7	211.2 ± 34.8	0.97

The study had sufficient power to detect a 10% difference in measured parameters. In summary, pharmacokinetics of i.v. and PO administered lomefloxacin were unaltered by acute pyelonephritis and were similar to published values in healthy volunteers.

Precision of true creatinine clearance and excretion in 1238 patients and normals with short collection periods. *A.Z. Győry and M. Ross, Department of Medicine, Sydney University, and Renal Medicine, Royal North Shore Hospital, St. Leonards, NSW, Australia.* A rapid, reproducible and practical technique of measuring GFR in clinical practice is still not readily available. We regularly measure 2 or 3 true creatinine clearances (C_{Cr}) under water restricted conditions with collection periods of 104 to 201 minutes and volumes of 101 to 201 ml. C_{Cr} were 116 ± 43 ± 1.2, 111 ± 41 ± 1.2, and 110 ± 38 ± 1.6 (M ± SE) for the first, second ($N = 1238$) and third ($N = 570$) periods. Precession from these duplicate measurements was 18%. C_{Cr} for males can be estimated from the formula:

$$C_{Cr} = \frac{(227 - \text{Age})\text{Wt}}{1416 \times P_{Cr}}.$$

(Age in years, wt. in kg and P_{Cr} in mmol/liter.) For females this has to be multiplied by 0.85. Creatinine excretion (mmol/day) was remarkably constant in 118 patients who had up to 9 repeat estimations (interval 3 days to 12 years) average CV was 11.5% for intra-patient variation. Normal ($N = 71$) C_{Cr} (ml/m/1.73 m^2) was 12 ± 27 ± 3.2 and varied with age. C_{Cr} as a percent corrected for age can be obtained from formula:

$$\frac{106 \times \text{GFR/SA}}{166 - \text{Age}}$$

Short collection periods without water loading offer a very suitable and reproducible way of rapidly measuring C_{Cr} if performed by trained nursing staff.

Membranous nephropathy: Prospective study of 51 patients. *N.M. Hay, R.R. Bailey, K.L. Lynn, and R.A. Robson, Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.* The outcome of 51 patients (33 males; mean age 50.8 ± 17.7, SD yr; 49 Caucasian, 2 Maori), aged 14 years and over with biopsy-proven membranous nephropathy (MN) was studied prospectively. The patients were enrolled between July 1, 1972 and June 30, 1991. Patients

with SLE were excluded. MN was secondary to drug therapy in 8 (gold 3, NSAID 3, penicillamine 1, captopril 1). All patients were HBsAg negative. In the majority of cases these patients did not receive immunosuppressive therapy. Forty-seven (92%) presented with the nephrotic syndrome, 8 (16%) with a plasma creatinine ≥0.15 mmol/liter and 28 (55%) had hypertension (BP > 150/90 mm Hg or on therapy). Mean follow-up was 5.5 years (range 4 months to 19 years). The 10-year cumulative renal survival was 78% and overall survival 66%. Six patients have entered the renal replacement program, and 6 have died of non-renal causes. Of the remaining 39, 8 have renal insufficiency, 27 hypertension and 22 proteinuria. Complete remission occurred in 17 (33%)—11 spontaneously, 4 following withdrawal of the offending drug, and 2 after immunosuppressive therapy. Poor prognostic indicators at presentation included renal insufficiency, need for antihypertensive therapy, pathological stage IV on renal biopsy and older age. MN is an indolent disease with a good chance of spontaneous remission. Aggressive treatment in most patients appears to be unwarranted.

A novel manifestation of aluminium toxicity: Prurigo nodularis. *M.A. Brown, C.R.P. George, S. Kalowski, R.A. Evans, and A.B. Corrigan, Concord Hospital, NSW, Australia.* We have observed three maintenance hemodialysis patients in whom aluminium overload has been associated with *prurigo nodularis* (PN). PN is characterized by chronic pruritis and widespread nodular skin lesions occurring mainly on the trunk. In two patients the rash developed after 8 months of hemodialysis, and in the third 10 months after recommencement of hemodialysis following failure of a renal transplant. In all three, the presence of aluminium-associated osteomalacia was demonstrated on bone biopsy. Apart from uremia, none had evidence of any other condition which has previously been causally associated with PN (such as insect bites, venom stings, or coeliac disease). Although treatment with oral and topical antibiotics, tricyclic anti-depressants, topical and intralesional corticosteroids produced little or no response; the itch in all patients responded to courses of desferrioxamine within one month, and the nodular lesions on the skin shortly thereafter. It is suggested that PN may be a manifestation of aluminium toxicity.

Normal values for overnight urinary albumin excretion (UAE) in healthy schoolchildren. *P. Henning, R. Ryall, S. Harris, and J. Penfold, Departments of Nephrology, Chemical Pathology and Endocrinology, Adelaide Children's Hospital, SA, Australia.* Elevated rates of UAE appear to predict diabetic nephropathy in adults, although the actual "pathological" cut-off has been derived retrospectively. In children the UAE rate may exceed control values yet not reach this predictive level for nephropathy. In addition, age-related normal data are needed in children. We measured UAE in a single overnight urine sample obtained from 888 children aged 5 to 17 years (420 females, 468 males). Albumin concentration was measured using an immuno-turbidometric method, and creatinine concentration using the standard Jaffe reaction. UAE was expressed as an excretion rate and as an alb/creat ratio, and analyzed as log transformed data. Each yearly age group was compared to every other using a *t* test. This analysis revealed a significant increase in UAE at 9 to 10 years, which was not seen at any other age. Female values were higher than male values up to this age.

	UAE rate (median) μg/min	95% Confidence limits
Female ≤9 years (N = 257)	2.07	0.5–8.6
Male ≤9 years (N = 240)	1.92	0.4–7.2
All subjects 10–17 years (N = 391)	2.96	0.6–13.9

When expressed as an alb/creat ratio UAE did not increase. The proportionate increase in albumin and creatinine excretion at 10 years of age correlates with the normal increase in BP in healthy children and may reflect the onset of adrenarche.

Ultrasonography (US) and computed tomography (CT) in the diagnosis of analgesic nephropathy. *M. Segasothy, S.A. Samad, A. Zulfigar, S.P. Menon, Z. Morad, and W. Shaariah, Departments of Medicine and Radiology, Universiti Kebangsaan Malaysia, Department of Nephrology, General Hospital, Kuala Lumpur, Malaysia.* The value of US in the diagnosis of renal papillary necrosis (RPN) in patients with impaired renal function has been established (Weber et al: *Nephron* 39:216–222, 1985). However, the role of CT in the diagnosis of RPN has not been well studied. In order to determine the value of CT in the diagnosis of RPN, we performed US and CT in patients who had consumed excessive quantities of analgesics (>1 kg) with normal or impaired renal function (serum creatinine ranging from 75 to 1094 μmol/liter). Criteria for the diagnosis of RPN by US and CT was renal papillary calcifications. As negative controls, 10 patients who had renal impairment due to glomerulonephritis but who had no history of analgesic abuse were studied. RPN was documented in 20 out of 40 patients by US and in 14 out of 40 patients by CT. In 11 patients both US and CT were positive. In 9 patients US was positive while CT was negative. In 3 patients CT was positive while US was negative. RPN was not documented in 17 patients and in any of the negative controls either by US or by CT. Prevalence of RPN was 50% using US and 35% using CT among analgesic abusers. Using US as a gold standard, sensitivity of CT was 55%, specificity of CT was 85%, positive predictive value was 78.6%, and negative predictive value was 34.6%. Percent agreement with CT and US was 70%. Cohen's Kappa statistic adjusting for chance agreement was 40%. Based on these results it is found that US yielded a higher percentage of positive cases of RPN. We recommend that in patients who have abused analgesics and who have impaired renal function, US is the investigation of choice for the diagnosis of analgesic nephropathy. CT could be performed when US is negative.

Thrombocytopenia in continuous hemodiafiltration (HDF) in intensive care acute renal failure (ICU ARF): 1988 to 1991. *K.J. Warr, G. Leslie, I.G. Jacobs, M. Webb, M.A.B. Thomas, P. Lee, and K.Y. Lee, Departments of Intensive Care, Renal Medicine and Haematology, Royal Perth Hospital, Perth, WA, Australia.* To determine the prevalence and associations of thrombocytopenia in ICU ARF treated with HDF, 144 consecutive cases were prospectively analyzed for daily platelet count ($\times 10^9$/liter), ICU admission diagnosis, disease severity (admission APACHE II score), heparin dose, effectiveness of HDF (Day 1 minus Day 4 creatinine, ultrafiltration volume), survival, and compared to 150 ICU non-ARF admissions, untreated by continuous heparin. Mild transient thrombocytopenia was consistently observed in the ARF group as a whole from commencement of HDF [Day 1 = 134 (107–207), median (quartiles), nadir at Day 4 = 103 (62–160), P = .0003, Wilcoxon] returning to baseline by Day 9 without specific therapy; no significant change in platelet count was seen in ICU non-ARF admissions. Multivariate analysis identified admission APACHE II score, trauma, post-cardiac bypass patients, and serum lactate (but not fall in creatinine, ultrafiltration volume) as independent determinants of platelet count. 5.8% of patients had platelet count <50 at start of HDF, which improved by day 2 to 50 to 150, without a transient secondary decline at Day 4. Only 2 cases of heparin-associated thrombocytopenia, confirmed by positive agglutination, were detected. Severe thrombocytopenia was managed by avoidance of heparin, pre-filter dilution, increased blood flow ± prostacyclin; low molecular weight heparin did not apparently alter rate of platelet recovery. Thrombocytopenia in HDF for ICU ARF is common, multifactorial, usually mild, maximal at Day 4 of HDF, and self-limited, without requirement for specific therapy. Patients with trauma, cardiac bypass and severe acidosis are at particular risk for this complication.

A comparison of laboratory and clinical factors in patients treated with hemodialysis (HD) and subsequent long-term hemodiafiltration (HDF). *P.G. Kerr, A. Argilés, B. Canaud, J.L. Flavier, and C. Mion, Department of Nephrology, Lapeyronie University Hospital, Montpellier, France.* Hemodiafiltration is purported to provide better cardiovascular stability for dialysis patients; other possible benefits of this therapy have not been well defined. We have compared serial treatment with HD and HDF in 20 stable patients over a period of 18 months. Dialysis parameters were: dialysate composition and flow, duration, and membrane used (polysulfone) were the same in the two periods except for the added convection of HDF (18–20 liters/session) and a higher tolerated blood flow in HDF (369 ± 24 vs. 348 ± 33 ml/min, $P < 0.005$). For both modes, ultrapure, sterile bicarbonate dialysate was used, and for HDF this was further ultrafiltered for use as infusate. Clinical parameters (pre- and post-dialysis BP, dry weight and weight loss per

session) were remarkably similar in the two treatment periods, indicating that stable patients do not benefit further from this therapy in terms of these factors. The clearance of urea was significantly improved with HDF, which was reflected in a higher Kt/V (1.55 ± 0.07 vs. 1.41 ± 0.05, $P < 0.001$) and lower TAC_{urea} (16.3 ± 1.0 vs. 19.3 ± 1.0 mmol/liter, $P < 0.0001$) without a significant change in the PCR. The clearance of beta-$_2$ microglobulin (β_2m) was also significantly improved by HDF compared to HD (% reduction in β_2m: 62.7% in HDF vs. 54.8% in HD, $P < 0.05$). There was a small difference in in-patient days favoring HDF which was of arguable significance. A cost analysis revealed no difference between HD and HDF (excluding initial capital costs), the cost of infusate production being offset by greater reuse of dialyzers. Thus, the benefit of HDF in stable dialysis patients is the improved clearance of small molecules and beta-2 microglobulin without increasing dialysis time. Further clinical benefits due to the improved clearance may only become apparent with longer follow-up.

Whole blood serotonin levels are markedly elevated in patients on dialytic therapy. *P.G. Kerr, A. Argilés, C.M. Mion, Department of Nephrology, Lapeyronie University Hospital, Montpellier, France.* The normal range for whole blood serotonin levels in CRF patients has not been defined. As serotonin may be implicated in platelet abnormalities, hypo- and hypertension and itch in dialysis patients, serotonin whole blood levels were measured in a group of patients with chronic renal failure. The levels were elevated in 12 patients with moderate (mean serum creatinine 335 ± 54 μmol/liter) chronic renal failure (270 ± 46 μg/liter) compared to 11 normals (163 ± 17 μg/liter $P < 0.05$; quoted normal range <300 μg/liter) but did not correlate with serum creatinine levels. There was a marked elevation in serotonin levels in dialyzed patients, including in hemodialysis (polysulfone, $N = 6$, 747 ± 234 μg/liter and cuprophane membranes, $N = 6$, 708 ± 198 μg/liter), hemodiafiltration ($N = 12$, 695 ± 130 μg/liter), and especially peritoneal dialysis ($N = 6$, 1148 ± 162 μg/liter). All results were significant ($P < 0.01$) compared to normals and compared to the non-dialyzed group ($P < 0.05$). The level of serotonin decreased during hemodialysis regardless of the membrane used. There was no correlation of serotonin levels with pruritus or hypertension, although there was a negative correlation with diastolic blood pressure. The reference range for serotonin whole blood levels needs to be broadened when considering dialyzed patients.

Beta-2 microglobulin (β2m) removal in hemodiafiltration (HDF) after reprocessing dialyzers with peroxyacetic acid. *P.G. Kerr, A. Argilés, B. Canaud, J.L. Flavier, C. Mion, Department of Nephrology, Lapeyronie University Hospital, Montpellier, France.* Reuse is widely practiced though its effects on the efficacy of removal of solutes and more recently proteins such as β2m are the subject of much debate. There is evidence to suggest that reprocessing with formalin, and/or bleach, maintains dialyzer performance. This study examines peroxyacetic acid use as the cleansing/sterilizing agent using Renatron® machines. It has been claimed that peroxyacetic acid does not remove any secondary membrane that may have formed, thus β2m removal may be subsequently impaired. We analyzed reuse in 24 patients using polysulfone membranes in a HDF unit over a two year period. The mean maximum number of uses achieved was 20.1 ± 0.5. Several factors considered clnically to influence the number of reuses achievable (Hb, WCC and platelet levels, ESR and fibrinogen and total protein level) were found not to affect the maximum number of uses obtainable. We then assessed prospectively the performance of 26 polysulfone dialyzers after peroxyacetic acid reprocessing up to 20 times, particularly with regard to their ability to remove β2m. The percent reduction in serum β2m was 71.8 ± 1.1% on first use, 72.7 ± 1.3% on 2nd, and 66.8 ± 2.2% on 20th use ($P < 0.05$ by paired t-test). On all occasions, the percent reduction in β2m exceeded that of urea (for example, 1st use % reduction in urea 69.9 ± 1.1%; 20th use 66.3 ± 2.4%). In addition we measured the % reduction in retinol binding protein (MW 21,000) and found it to be moderately reduced by the 20th use (11.2 ± 2.4% vs. 18.6 ± 2.1%, $P < 0.05$). Thus, we can report that polysulfone membranes reprocessed with peroxyacetic acid used for HDF up to 20 times exhibit maintained high level removal of compounds beyond a MW of 12,000. Any secondary membrane formation that occurs appears not to influence the subsequent removal of β2m. Thus we would recommend the use of peroxyacetic acid for reprocessing dialyzers in a safe and efficacious manner.

The accuracy of Kt/V estimations in hemodiafiltration (HDF) using percent reduction in urea (PRU): Incorporation of urea rebound. *P.G. Kerr, A. Argilés, B. Canaud, J.L. Flavier, C. Mion, Department of Nephrology, Lapeyronie University Hospital, Montpellier, France.* The estimation of Kt/V by utilization of the pre- and post-dialysis urea levels [PRU and $\ln(U_{pre}/U_{post})$] provides a simple, quick technique that can be applied at the bedside. However, the accuracy of such techniques has been questioned. One possible reason for this inaccuracy may be the post-dialysis rebound in serum urea levels that is frequently observed. We assessed the urea rebound at 30 minutes post-dialysis in 34 hemodiafiltered patients and compared the calculation of Kt/V using this urea level with that using the immediate post-dialysis level. The results obtained using 2 formulae incorporating PRU and also the $\ln(U_{pre}/U_{post})$ were then compared to the Kt/V calculated by urea kinetic modelling (UKM), also utilizing both serum urea levels. The degree of urea rebound observed was large, 21.4%, as was the creatinine rebound, 26.0%, being a reflection of the short duration, rapid flux dialysis. The formulae for calculation of Kt/V all significantly correlated with Kt/V by UKM (r values 0.799–0.959, all $P < 0.0001$) but all gave results significantly different to Kt/V by UKM ($P < 0.001$ by paired t-test). The formula of Jindal (0.04PRU-1.2) overestimated Kt/V by 0.19 ± 0.03 or 0.16 ± 0.01 for immediate or delayed urea sampling, respectively; whereas that of Basile (0.023PRU-0.284) underestimated by 0.14 ± 0.03 or 0.07 ± 0.02. The $\log_e$ formula underestimated Kt/V by a greater degree. In all cases the delayed urea sampling provided estimated Kt/V values closer to the UKM derived Kt/V values. For assessment of Kt/V by these formulae, or by UKM utilizing single pool kinetics, the urea rebound is too large to ignore in the setting of short duration, rapid flux dialysis.

Age and parathyroid (PTH) suppression on dialysis. *A. St. John, K. Hoad, P. Lee, K.J. Warr, M.A.B. Thomas, Departments of Biochemistry and Renal Medicine, Royal Perth Hospital, Perth WA, Australia.* To examine determinants of PTH concentrations in non-parathyroidectomized patients on maintenance dialysis, data on calcium-phosphate metabolism, dialysis duration (Dx months) and therapy (including $Ca.CO_3$ g/day) were prospectively recorded six-monthly between 1988 to 1991 in 500 samples from 142 patients. Intact PTH levels by immunochemiluminometry (N:0.5–5.5 pmol/liter) were ≥10 pmol/liter in 73% of samples (median 42, range 0.6–305), whereas alkaline phosphatase (N:40–135 U/liter) was elevated in only 25% of samples (98,0–890 U/liter). Patients with ≥3 measurements of PTH were divided into: (1) High: PTH #1 >10 pM and stable/increasing over time ($N = 18$); (2) Decreasing: PTH #1 ≥10 and ≥50% fall over time, ($N = 14$), and (3) Low: median PTH <10 ($N = 14$).

	High	Decreasing	Low	P
PTH #1	67 (23–240)	62 (21–305)	5 (0.7–16)[a]	0.00 vs. H,D
Age *years*	51 (38–74)	51 (34–67)	67 (19–74)[a]	0.00 vs. H,D
Dx *months*	51 (25–203)[a]	43 (21–162)	45 (26–89)	0.00 vs. D,L
$CaCO_3$	1.7 (0–4.5)	4.5 (1.39–)[a]	1.5 (0.4–9)	0.04 vs. H

By multiple regression, elevated PTH was independently associated with duration of dialysis ($r = 0.48$, $P = 0.006$) and serum PO_4 ($r = 0.49$, $P = 0.005$), the extent of fall in PTH correlated with lower serum urea ($r = -0.31$, $P = 0.03$), and low PTH levels correlated with increasing age ($r = -0.48$, $P = 0.04$). Clinically-indicated bone biopsies demonstrated pure hyperparathyroidism in 4/4 High patients, mixed HPTH/osteomalacia in 2/3 Decreasing patients and adynamic bone disease in 2/2 Low patients. Biochemical hypereparathyroidism is very prevalent with evolution over time on dialysis, affected by PO_4 control. Relative "hypoparathyrodism" is a feature of the elderly dialysis patient and may predispose to adynamic bone disease.

Calcium alginate versus aluminium hydroxide in maintenance hemodialysis. *D.C.H. Harris, L. Yuill, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* The risk of aluminum toxicity has stimulated a search for safe alternatives to aluminium-containing phosphate binders. In preliminary studies from Germany, calcium alginate, a natural aluminum-free polyuric acid, has been reported to be an effective and safe treatment for uremic hyperphosphatemia in dialysis

patients. Treatment with calcium alginate (C, max 8.4 g/day) was compared to that with aluminium hydroxide (A, max 5.4 g/day) in 17 chronic hemodialysis patients in a 12 month randomized, cross-over trial. After 5 and 6 months of treatment, serum phosphate was no different with C compared to A (for example 6 months, 1.75 ± 0.15 vs. 1.68 ± 0.09 mmol/liter, $\mu \pm$ SEM). Plasma aluminium was significantly lower in C from 2 months (1.54 ± 0.26 vs. 3.04 ± 0.62 μmol/liter, $P < 0.01$). Plasma aluminum after 40 mg/kg IVI desferrioxamine tended to be lower in C (for example, 6 months, 4.83 ± 0.68 vs. 8.16 ± 1.48 μmol/liter, NS). Serum calcium, magnesium, alkaline phosphatase and immunoreactive parathyroid hormone were no different during the treatment periods. For adequate phosphate control, an extra phosphate binder was required with both C and A (Mylanta II, 1.1 ± 0.3 vs. 0.4 ± 0.3 tablets/day, NS). C was well tolerated, and mild diarrhea and hypercalcemia in one patient each were the only side effects. Of 12 patients who completed both arms of the study, 5 preferred to take A, 5 preferred C, and 2 had no preference. In summary, calcium alginate is a safe, well-tolerated and effective phosphate binder, but must be used with another agent in the majority of patients receiving maintenance hemodialysis.

Intestinal failure secondary to sclerosing peritonitis in patients on chronic peritoneal dialysis—A role for home TPN? *A. Chrysostomou, G. Becker, R.J.S. Thomas, D.M. Russell, Department of Nephrology and Clinical Nutrition Service, The Royal Melbourne Hospital, Victoria, Australia.* Sclerosing peritonitis is a recognized major complication associated with chronic peritoneal dialysis (PD). In advanced cases, chronic intestinal obstruction results in malnutrition and the need for TPN. Between 1979 and 1991, 311 patients at The Royal Melbourne Hospital received chronic PD, and 6 patients, aged 33 to 61 years, developed sclerosing peritonitis (incidence 1.9%). The median duration of PD until intestinal failure was 4.5 years (range 1.5–12.5 years). All had prior episodes of peritonitis with a mean of 1 infection episode per 10 months of dialysis (range 1/27 months to 1/4.5 months). Five out of six patients had multiple episodes of peritonitis. Polymicrobial cultures were obtained from the peritoneal effluent of all patients, although coagulase negative *staphlococcus* was the predominant organism. Five out of six patients have died of intestinal failure (mortality 83%), and 1 patient has been on Home TPN for 9 months. The early recognition of sclerosing peritonitis in patients on chronic peritoneal dialysis is paramount to prevent intestinal failure, and a change from PD to hemodialysis is recommended. Home TPN in a patient on hemodialysis is both a technical and metabolic challenge.

Changes in blood rheology during hemodialysis. *B.I. Shand, A.L. Buttimore, K.L. Lynn, L.J. Chisholm, C.M. Stanley, Department of Nephrology, Christchurch Hospital, Christchurch, New Zealand.* We investigated the changes in indices of whole blood viscosity in 7 home hemodialysis (HD) patients during two 5 hour standardized HDs, using either flat plate or hollow fiber Cuprophan® membrane dialyzers. Blood samples for hematological, viscometric and filtration studies were collected at baseline, 2 minutes, and then at hourly intervals. HD using either membrane caused a similar pattern of changes with an increase in mean hematocrit, whole blood and plasma viscosity, and whole blood filtration, while red blood cell (RBC) deformability did not change. HD with a hollow fiber dialyzer was associated with significant and rapid changes in plasma viscosity ($P < 0.02$) and whole blood filtration ($P < 0.05$). Three patients developed marked rheological changes with both dialyzers. The rise in whole blood and plasma viscosity with either membrane both correlated with increased hematocrit ($r = 0.90$ and 0.89, respectively). The cause of the reduction in whole blood filtration is unknown and occurred independently of the viscosity increases and was also not the result of impaired RBC deformability. We conclude that HD may cause significant changes in blood rheology, mainly as a result of hemoconcentration. In the case of Cuprophan® dialyzers, the time frame and magnitude of these rheological changes is influenced by the membrane configuration.

Low dose subcutaneous (s.c.) vs. intravenous (i.v.) recombinant human erythropoietin (EPO) in maintenance hemodialysis (HD) patients. *P.G. Barclay, E.R. Fischer, D.C.H. Harris, Departments of Renal Medicine and Pharmacy, Westmead Hospital, NSW, Australia.* Although s.c. EPO has been reported to correct anemia in HD patients at lower doses than i.v. EPO, these trials involved high dose EPO and did not control for the time-related fall in dose, which has been shown with low dose EPO. Therefore, a double crossover study was performed to determine the effect of route of administration of EPO on hemoglobin (Hb) response. EPO dose was kept constant unless Hb rose above 100 g/liter, when the dose was reduced until Hb was 90 to 100 g/liter. Initial EPO dose was 77.5 ± 10.7 (mean ± SEM) U/kg/week. Thirteen (4 male) stable HD patients, who had been on i.v. EPO for 15.3 ± 2.6 months, were given EPO i.v. for 12 weeks (IVI#1), then s.c. for 24 weeks and then i.v. (IVI#2) for a further 24 weeks (as yet IVI#2 has not been completed). Iron status and other factors known to modify EPO response were kept constant. Two patients who developed iron deficiency during the study have been excluded from analysis. The mean Hb (g/liter) during the last 3 months and final Hb of each arm was no different.

	Last 3 months			
	Last 3 months		s.c.-IVI	Final
IVI#1	87.4 ± 2.2	NS	1.3 ± 2.8	89.4 ± 3.9
SC	89.4 ± 2.8			88.1 ± 3.4
IVI#2	86.5 ± 3.3	NS	2.8 ± 3.0	87.6 ± 3.8

Mean Hb was higher on i.v. EPO in 6 patients (by 5.5 ± 1.9 g/liter), and on s.c. EPO in 5 patients (by 10.7 ± 4.2 g/liter), and by >5 g/liter in 3 patients while on i.v. and in 4 while on s.c. EPO. In conclusion, although there is no overall advantage in administering low dose EPO subcutaneously or intravenously, the most effective route of administration should be determined for each patient.

Comparison of 3 immunosuppressive regimens in cadaver renal transplantation: Long-term cyclosporine (CsA), short-term CsA followed by azathioprine and prednisolone, and azathioprine and prednisolone without cyclosporine. An 8 year follow-up. *Australian Collaborative Trial Committee.* A study comparing the three treatment regimens commenced in 1983. 489 patients were entered into the study, as shown in Table 1.

Total	Conv. tmt	Long-term CsA	3 month CsA then conv.
489	158	166	165
Dead	31[a]	17	20
Lost to f/u.	1	2	1
On dialysis	38	42	39
Died with a functioning kidney	22[a]	11	14
Alive with no change in Tmt	89	105	101

[a] $P = <0.05$

Patients assigned to long-term cyclosporine (CsA) or short-term CsA had similar 12 month actuarial survival (98.4 vs. 96.4) and graft survival (83.9 vs. 82.1). Patients assigned to azathioprine and prednisone with the optional use of ATG had a significantly poorer survival rate of 91.3% ($P < 0.05$) at 12 months due to increased deaths secondary to cardiovascular disease. Graft survival did not differ significantly. At 96 months, long-term CsA and short-term CsA showed a 91% and 88% patient survival, respectively, while conventional therapy patients continued to have a significantly worse survival ($P < 0.05$). Graft survival at 96 months was 66% on long-term CsA, 62% on short-term CsA and 54% in the conventional arm ($P > 0.05$). Serum creatinine was 169.1 ± 87 in long-term CsA, 129.65 ± 53 in short-term CsA ($P < 0.05$) and 153.4 ± 84 μmol/liter in conventional treatment at 96 months. At 96 months, graft survival is not significantly different between the 3 arms of the study. Patient survival continues to be better in the CsA arms.

CD-25 (anti-IL-2R) monoclonal antibody (mAb) treatment (Rx) depletes IL-2R+ (p55) mononuclear cells (MNC) from cardiac but not concurrent rat renal allografts. *W. Hancock, J. Kupiec-Weglinski, H.*

Ueda, N. Tilney, Departments of Pathology and Immunology, Monash Medical School, Melbourne, Victoria, Australia, and Surg Res Lab, Harvard Medical School, Boston, Massachusetts, USA. Clinically, CD25 mAb Rx of acute renal Tx rejection has proven less satisfactory than anticipated from rodent studies. CD25 mAb Rx (ART-18, 300 μg/kg/d × 10 days, i.v.) was previously shown to deplete IL-2R+ MNC from rat cardiac allografts (LBNF1→LEW) but not from kidney grafts. However, if normal renal function was maintained by omitting native nephrectomy (Nx), mAb *did* deplete IL-2R+ MNC, suggesting suppression of host ADCC by uremia. We now asked whether uremia would therefore inhibit depletion of IL-2R+ MNC from both heart and kidney allografts in dual Tx recipients. Accordingly, LEW rats with Nx received LBNF1 heart *and* kidney grafts plus CD25 mAb Rx. Control rats (2–4/group) received single allografts and either no Rx; CD25/no Nx, CsA 15 mg/kg/d × 10 days, or low CsA ± CD25 mAb × 10 days. Graft function was assessed by creatinine (Cr) or ventricular contractions. Rats were sacrificed on day 7 post-Tx for histology or followed until death. Untreated Nx dual recipients rejected their cardiac grafts at 7 ± 1 day and died of uremia at 9 ± 1 day. CD-25 mAb Rx dual recipients showed excellent cardiac function but were uremic from day 7 and died at 13 ± 2 days. Analysis of intragraft IL-2R+ MNC (% total leukocytes) at 7 days post-Tx showed:

	Cr	Heart	
	mg/dl	% IL-2R+	Survival
(1) Dual Tx			
No Rx	5.8	16.2 ± 1	7 ± 1 days
CD-25 Rx	3.2	0.4 ± 0.3[a]	>13[c]
(2) Single Tx			
No Rx	5.8	14.6 ± 3.2	7 ± 1
CD-25 Rx	3.2	0.2 ± 0.1[a]	21 ± 2[a]
CD-25/no Nx	<1	0.2 ± 0.1[a]	21 ± 2[a]
CsA Rx	<1	0.1 ± 0.1[a]	>60[a]
low dose CsA	2.8	13.6 ± 2.7	7 ± 1
CD25/low CsA	1.7	0.1 ± 0.1[a]	>45[a]

	Kidney	
	% IL-2R+	Survival
(1) Dual Tx		
No Rx	17.3 ± 4.2	9 ± 1 days
CD-25 Rx	15.2 ± 5.2	13 ± 2
(2) Single Tx		
No Rx	16.6 ± 3.6	10 ± 4
CD-25 Rx	15.1 ± 5.3	23 ± 7[c]
CD-25/no Nx	0.8 ± 0.6[a]	23 ± 7[c]
CsA Rx	0.2 ± 0.1[a]	>60[a]
low dose CsA	14.4 ± 4.3	16 ± 2
CD25/low CsA	2.3 ± 1.1[b]	>45[a]

[a] $P < 0.001$, [b] $P < 0.005$, [c] $P < 0.01$ cf. to respective no Rx group

These data show that uremia inhibits CD25 mAb depletion of IL-2R+ MNC from rejecting renal but not corresponding cardiac allografts. Moreover, adding even sub-therapeutic doses of CsA to the CD25 mAb Rx protocol restores the therapeutic effect of CD25 mAb Rx in renal Tx recipients and synergistically prolongs graft survival.

Diltiazem in renal transplant recipients receiving cyclosporin A (CsA). *Anastasia Chrysostomou, Rowan Walker, Timothy Mathew, Graeme Russ, Anthony d'Apice, Pricilla Kincaid-Smith, Royal Melbourne Hospital, Victoria, and The Queen Elizabeth Hospital, South Australia, Australia.* Of 113 CsA treated primary renal allograft recipients, 60 were randomized to receive either standard therapy (ND), and 53 received standard therapy plus diltiazem (D). There was no difference in CsA blood levels between ND and D at all intervals between 3 and 24 months, yet the D required 35% less CsA than the ND group (measured at 12 months). At all intervals to 24 months follow-up there was no difference in blood pressure, renal function as measured by serum creatinine (mean ± SD) (ND; 0.12 ± 0.02 mmol/liter, D; 0.10 ± 0.03 mmol/liter at 24 months), or the number of grafts lost in the 2 groups (ND; 4 lost, D; 3 lost). There was no significant difference in the total number of rejection episodes in the 2 groups (ND; 89 episodes, D; 71 episodes). However, the severity of rejection episodes was greater in the ND group as evidenced by a significant difference in the usage of orthoclone OKT3 (ND; 17 courses OKT3, D; 8 courses OKT3, $P < 0.05$). Of biopsy proven episodes of rejection there was also significantly more episodes of vascular rejection (ND; 14 episodes, D; 3 episodes, $P = 0.005$). The incidence of primary non-function was less in the D group (ND; 11 patients, D; 4 patients, $P = 0.05$). The D group required 35% less CsA than the ND group, with no change in graft outcome. Diltiazem with CsA resulted in significantly less severe rejection episodes and in particular fewer episodes of vascular rejection.

Lymphocytes from a highly sensitized dialysis patient synthesize a monoclonal antibody to the HLA-B5 cross-reactive antigen group (CREG). *D.A. Power, B.K. Weber, I. Al Muzairai, M.C. Jones, Departments of Nephrology and Clinical Immunology, St. Vincent's Hospital, Fitzroy, Victoria, Australia.* Antibodies in sera from highly sensitized dialysis patients react with T lymphocytes from ≥50% of normal donors. Some studies have suggested that this is due to the presence of antibodies against public HLA specificities (cross-reactive groups or CREG's). We have produced a human monoclonal antibody of class $IgG_1\lambda$ (called FLP) to HLA antigens of the −B5 CREG by Epstein-Barr virus transformation and somatic cell hybridization of lymphocytes from a highly sensitized patient (FW). This antibody was not cytotoxic, even in AHG-CDC. Assay of FLP against 57 cell lines (29 normal peripheral blood lymphocytes and 28 lymphoblastoid cell lines) showed a strong correlation between reactivity of FLP by flow cytometry and the presence of members of the HLA-B5 CREG. When attempting to correlate the specificity of the monoclonal antibody FLP with the reactivity of serum samples against normal peripheral blood lymphocytes by flow cytometry, it was apparent that this antibody was only one of a number present in sera from the patient FW. A rabbit anti-idiotypic antibody was then raised against FLP; it inhibited binding of IgG antibodies in serum obtained from FW to cells which reacted with FLP and, also, binding to a cell which did not. These results demonstrate that: (1) B-cells from highly sensitized patients produce antibodies to CREG's, (2) IgG antibodies from these patients may be non-cytotoxic, and (3) alloantibodies against distinct specificities may possess similar idiotypes.

T-cell antigen receptor Vα gene expression in a rejecting renal allograft. *M.C. Falk, Y. Zhang, J.R. Chapman, L.P. Roy, J.F. Knight, Renal Research Laboratories, The Children's Hospital, Camperdown and Renal Unit, Westmead Hospital, Westmead, NSW, Australia.* Rejection of a renal allograft by the host is usually characterized histologically by the infiltration of mononuclear cells. Histochemical staining has demonstrated that the infiltrate is predominantly composed of T-cells. This observation provides the rationale for the use of OKT3, a pan-T-cell monoclonal antibody directed at CD3, to treat rejection. The majority of T-cell antigen receptors (TCR) comprise two distinct glycoproteins, termed α and β. These molecules have variable (V) regions analogous to immunoglobulin V regions, which confer antigen specificity. In humans the gene for the TCR Vα chain is located at chromosome 14q11-12. To date, 48 different Vα genes have been described, which have been assigned to 18 families on the basis of sequence homology. If T-cells mediating rejection belonged to a specific Vα or Vβ family or families, it would be possible to design more specific strategies directed at those particular cells. To explore this hypothesis, we have synthesised 5′ oligonucleotide primers for each of the 18 TCR Vα families and a 3′ primer specific for the Cα (constant region) gene. Messenger RNA was extracted from peripheral blood mononuclear cells (PBMC), from a "normal" kidney (the unaffected pole of a kidney removed for Wilms' tumor) and from an allograft undergoing acute rejection, and converted to cDNA by reverse transcription. The polymerase chain reaction was used to detect the presence of TCR Vα gene expression in these samples and to characterize the spectrum of Vα families being expressed. In our preliminary experiments, expression of 17/18 TCR Vα genes was demonstrated in PBMC. In the "normal" kidney several faint bands were seen suggesting the presence of a few passenger lymphocytes. In the rejecting renal allograft expression of

15/18 TCR Vα genes was readily detected, suggesting that, at least in this specimen, a polyclonal T-cell infiltrate was occurring. We plan to use this method to analyse evolution of the T-cell infiltrate during the process of cellular rejection.

Long-term reduction in bone mineral density following renal transplantation. *H.D. McIntyre, B. Menzies, D.A. Perry-Keene, R. Rigby, I.R. Hardie, Renal Transplant Unit and Department of Endocrinology, Princess Alexandra Hospital, Queensland, and Department of Endocrinology, Royal Brisbane Hospital, Australia.* Serial measurements of serum and urine markers of bone metabolism and of forearm bone density by dual photon absorptiometry were performed in 22 renal transplant recipients at the Princess Alexandra Hospital in 1986. Patients were randomized to receive (1) cyclosporine alone (CsA, $N = 9$), (2) cyclosporine for 3 months followed by azathioprine-prednisone ($N = 3$) or (3) long-term azathioprine-prednisone ($N = 9$). As no reduction in bone mineral density (BMD) was noted in the first six months, groups 2 and 3 were considered together (Aza + Pred group, $N = 12$). Overall, mean ± SD BMD fell from 1.39 ± 0.21 g/cm^2 at transplantation ($N = 21$), to 1.15 ± 0.24 g/cm^2 at 36 months ($N = 19$), and 1.19 ± 0.25 g/cm^2 at 60 months ($N = 14$) post-transplant (ANOVA, $P < 0.01$). Similar reductions in BMD over 36 months were seen in the CsA group (17%, $P < 0.05$) and the Aza + Pred group (19%, $P < 0.05$). Small patient numbers made subgroup analysis difficult at 60 months. The rate of loss of BMD correlated with the dose of methylprednisolone (MP) received for acute rejection episodes ($r = 0.68$, $P < 0.01$) and the total (MP + oral) prednisolone dose ($r = 0.64$, $P < 0.05$). No significant correlations were observed between the rate of loss of BMD and serum alkaline phosphatase or urinary hydroxyproline/creatinine or calcium/creatinine ratios determined shortly after or at 12 months after transplantation. Serum alkaline phosphatase fell post-transplant in patients treated with Aza + Pred, but not in the CsA group. These results demonstrate significant loss of forearm bone mineral with long-term follow-up after renal transplantation, and suggest that patients treated with cyclosporine without maintenance prednisolone also risk osteopenia.

Hypercalcemia and hyperparathyroidism following renal transplantation. *C.M. Hawley, H.D. McIntyre, R. Grudenich, B. Menzies, E. Maddens, I. Hardie, G. Briggs, R. Rigby, J. Petrie, M. Suranyi, L. Hartley, Renal Transplant Unit, Department of Endocrinology, Department of Surgery, Princess Alexandra Hospital, Woolloongabba, Australia.* We performed a retrospective study of all patients with functioning renal allografts attending our renal transplant clinic, to evaluate the incidence and natural history of, and factors responsible for hypercalcaemia, as well as the effect of hypercalcemia on allograft function and bone status. In the 166 patients with first allografts followed for a period from 1 to 19 years, we found that the incidence of hypercalcemia (Ca^{++}, Corr > 2.7 mmol/liter) peaked at 32% at 8 months post-transplantation and decreased progressively from that time reaching approximately 10% at 10 years. There was a linear fall in the mean plasma calcium concentration with increasing time post-transplant ($r = 0.86$, $P < 0.01$). Hypercalcemia was not associated with impairment of allograft function, and the type of immunosuppressive therapy received did not appear to influence the incidence of hypercalcemia. In hypercalcemic patients there was a significant correlation between ionized calcium and parathyroid hormone (PTH) ($r = 0.55$, $P < 0.05$), ionized calcium and osteocalcin ($r = 0.61$, $P < 0.01$), PTH and osteocalcin ($r = 0.71$, $P < 0.01$), alkaline phosphatase (ALP) and PTH ($r = 0.55$, $P < 0.05$), and ALP and osteocalcin ($r = 0.51$, $P < 0.05$). The hypercalcemic patients are being further evaluated with detailed symptom analysis, assessment of fracture prevalence, bone densitometry, measurement of total body calcium, quantification of vascular calcification, and bone histomorphometry.

Parathyroidectomy (PTE) + graft dysfunction in renal transplant (TP) recipients. *M.G. Suranyi, C. Hawley, R. Rigby, L. Hartley, J. Petrie, J. Burke, I. Hardie, Renal Unit and Department of Surgery, Princess Alexandra Hospital, Brisbane, Australia.* Subtotal-PTE post-TP was reviewed, and 13 cases (1.5–79 months post-TP) found at PAH in the last 3 years. The indications were hypercalcemia, bone pain and active hyperparathyroidism. Although renal function was stable despite ↑ [Ca], prior to PTE, 5/13 had episodes of renal impairment immediately post-PTE. All patients received oral Vit D, IV or oral Ca and Mg if required, post-PTE. Of the 5/13 patients with acute renal impairment after PTE, post-op hypercalcemia occurred in 4/5. In the 1/5 patients with acute renal impairment after PTE, who was non-hypercalcemic, only minor serum creatinine (Creat) rise was found, which returned to baseline with no specific therapy. In the 4/5 hypercalcemic patients acute rejection was diagnosed clinically, and steroid anti-rejection therapy was administered in 3 with a beneficial response. Only one had a renal TP biopsy, which confirmed acute cellular rejection. The fourth patient received only an increase in the maintenance oral steroid dose and renal dysfunction and hypercalcemia persisted. In each case with hypercalcemia and graft dysfunction, the ↑ Creat level was found to closely parallel the post-PTE [Ca], whereas there was no detectable relationship between [Ca] and Creat prior to PTE. Vit D, PTH and [Ca] may significantly effect both kidney function and the recipient immune system. The ↑ Creat post-PTE correlated with ↑ Ca levels post-PTE, suggesting that ↑ [Ca] should be avoided in the post-PTE period in TP recipients. PTE alters Ca, PTH and Vit D levels, all important to immune cells, or may interfere with the effect of immunosuppressive drugs.

In vivo effect of losartan (DuP 753) on AT_1 angiotensin II receptors (Ang II) in rat kidney and adrenal. *J. Zhuo, P.J. Harris, and F.A.O. Mendelsohn, Departments of Physiology and Medicine, University of Melbourne, Austin Hospital, Heidelberg, Victoria, Australia.* Our objective was to investigate in vivo effect of AT_1 receptor antagonist, losartan, on Ang II receptor binding in rat kidney and adrenal by in vitro autoradiography. Rats ($N = 4$ each group) were injected with saline or losartan (1, 3, 10 mg/kg, BW) via the tail vein, killed by decapitation, and the kidneys and adrenals removed at 1 hour, 2 hours, 8 hours, and 24 hours after injection. Cryostat sections of tissues were incubated in phosphate buffer containing ^{125}I-[Sar^1, Ile^8] Ang II with (non-specific binding) or without (total binding) unlabeled Ang II. The slides were exposed to X-ray film and the autoradiographs were analyzed by computerized densitometry. In the kidney, losartan caused dose- and time-dependent inhibition of the AT_1 binding at glomeruli, proximal convoluted tubules, and the inner stripe of the outer medulla. The 1 mg/kg dose resulted in 50% inhibition and the 10 mg/kg dose produced 90% inhibition ($P < 0.001$). These effects peaked at 1 hour, and persisted for 24 hours. In the adrenal, losartan caused 60% and 40% inhibition of the AT_1 binding at 1 hour ($P < 0.001$), and the inhibition fell to 30% and 18% at 24 hours in the cortex and medulla, respectively. These results indicate that losartan and/or its active metabolite, EXP 3174, selectively block AT_1 Ang II receptors in the kidney and adrenal and may provide a new therapeutic agent in treating renal disorders associated with activation of intrarenal renin-angiotensin system.

Effects of insulin-like growth factor-I in rats with acute renal failure. *A.A. Martin, C.G. Gillespie, S.J. Hazel, and L.C. Read, Child Health Research Institute, North Adelaide, South Australia, Australia.* Insulin-like growth factor-I (IGF-I) may be therapeutically beneficial in the treatment of acute renal failure by increasing renal blood flow, glomerular ultrafiltration coefficient and glomerular filtration rate (GFR), stimulating regeneration of renal tubule cells in acute tubular necrosis and ameliorating the catabolic state. We examined the efficacy of IGF-I in improving GFR and enhancing growth in rats with prerenal ARF. Acute renal failure was produced in male rats, 250 g body weight, by clamping both renal arteries for 45 minutes. IGF-I, or its variants des(1–3)IGF-I or LR^3-IGF-I, all at dose rate 2 mg/kg/day, were administered for 7 days via s.c. osmotic pumps. Vehicle alone was administered s.c. to the control group. ^{51}CR-EDTA was infused by osmotic pump i.p. to allow daily calculation of GFR from serum and urine samples. Rats were held in metabolic cages for daily measurements of body weight, food and water intake, and 24 hour urine output. GFR was decreased by 60 to 70% after renal ischemia and was unaffected by IGF peptide treatment. GFR returned to normal levels on day 7 of treatment in all four groups of rats. Daily urine output tended to be lower in all three peptide-treated groups than in the control group, while water intakes were similar between groups; fluid retention averaged 13 ± 0.7 ml/day (IGF-I), 13 ± 1.1 ml/day [des(1–3)IGF-I] and 14 ± 1.1 ml/day (LR^3-IGF-I), significantly above that in controls (10.3 ± 0.6 ml/day; $P < 0.05$). Body weight gain over the 7 days was increased in all 3 peptide groups (averaging 4.6 ± 0.6 g/day (IGF-I), 4.6 ± 0.7 g/day [des(1–

3)IGF-I], 5.1 ± 0.6 g/day (LR^3-IGF-I) vs. 1.9 ± 0.6 g/day in controls; $P < 0.05$), while food intake was similar between groups. Fluid retention and body weight gain were significantly correlated ($R^2 = 0.62$, $P < 0.001$). IGF-I and its variants were ineffective in improving GFR in acute renal failure while causing significantly increased fluid retention and a related body weight gain. No increased potency of the IGF variants over the native IGF-I molecule was observed. This study was supported by the Australian Kidney Foundation.

Evidence for activation of tubuloglomerular feedback (TGF) after nephrectomy (Nx) in humans. *C.A. Pollock and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, NSW, Australia.* Data from our previous micropuncture experiments in rat models of renal ablation have demonstrated an increase in TGF activity. This may have important clinical implications, since manipulations which impair the TGF response may theoretically result in the long-term deterioration of renal function by promoting hyperfiltration in individual nephrons. We therefore devised a means for determining the role of TGF in GFR regulation following Nx in normal humans. Six normal subjects (32–62 years) underwent assessment of GFR (measured as creatinine clearance) prior, and 4 to 6 weeks subsequent to Nx, performed for the purpose of living related transplantation. The contribution of TGF to the regulation of GFR both before and after Nx was assessed by determining the baseline steady state GFR, and then the GFR with the effect of TGF activity removed. This was achieved by the administration of a 1 mg dose of bumetanide (with quantitative replacement of salt and water losses), which blocks the afferent arm of the feedback response. The extent to which GFR rose after bumetanide was taken as a measure of underlying TGF activation, and this parameter was compared in the subjects in their pre- and post-Nx state. Baseline GFR prior to Nx was 115 ± 7 ml/min, which increased by 9.5 ± 2.5% to 126 ± 9 ml/min following blockade of TGF. Four to six weeks following Nx the baseline GFR was 83 ± 8 ml/min, that is, 72% of the 2 kidney GFR. After administration of bumetanide, the GFR increased by 15.6 ± 3.1% to 96 ± 9 ml/min, a significantly greater fractional rise than that demonstrated prior to nephrectomy ($P < 0.025$). These results indicate an increase in tonic TGF activity in the uninephrectomized state, compared with that demonstrated prior to Nx. Overall, these results suggest that the hyperfiltration response following unilateral Nx is limited by an appropriately activated TGF system. It follows that clinical maneuvers which blunt TGF, such as loop diuretic therapy and high protein feeding, may cause long-term deterioration in renal function as a pathological consequence of an excessively high filtration rate in the remaining nephrons.

Skeletal muscle metabolism in uremic rats. *C.H. Thompson, G.J. Kemp, Y. Green, G.K. Radda, and J.G.G. Ledingham, MRC Biochemical and Clinical Magnetic Resonance Unit, John Radcliffe Hospital, Oxford, United Kingdom.* The etiology of uremic myopathy is poorly understood. Clinical studies suggest impaired oxidative and anaerobic metabolism. Cation transport is abnormal with decreased Na/K ATPase activity and elevated intracellular sodium. The effect of uremia on skeletal muscle metabolism of the rat was examined using ^{31}P magnetic resonance spectroscopy. Three weeks following either a 5/6 nephrectomy or a sham operation ($N = 5$ in each group), Wistar rats were placed in a 7 T magnet and the sciatic nerve stimulated at 2 Hz for 10 minutes. Analysis of spectra taken at rest, during exercise and recovery allowed calculation of intracellular pH and the relative concentrations of phosphocreatine (P_{Cr}), inorganic phosphate (Pi) and ATP. [ADP] (μmol/kg wet weight) was calculated from the creatine kinase equilibrium.

	Plasma		Muscle			
	mmol/liter		Rest	Exercise		Recovery $P_{Cr}(t_{1/2})$
	Pi	creat	Pi/ATP	pH	[ADP]	*min*
Uremia	2.4[a]	0.13[a]	0.43[a]	6.96	70	0.86
Control	2.1	0.04	0.60	7.01	53	0.68

[a] $P < 0.05$; unpaired *t*-test

There was a significant reduction in the resting intracellular Pi despite an elevation in extracellular Pi, probably due to a reduction in the activity of the membrane Na/Pi cotransporter on account of a reduced sodium gradient. Despite anemia and uremia, there were no significant metabolic abnormalities accompanying this substantial reduction of GFR. In particular, the similar changes during exercise and recovery in controls and in these uremic animals imply that at this level of renal impairment there is no mitochondrial dysfunction.

^{13}C NMR studies of proximal tubular glycine metabolism. *G.J. Cowin, D.A. Willgoss, P.A. Stewart-Richardson, Z.H. Endre, University of Queensland, Department of Medicine, Royal Brisbane Hospital, Herston, Australia.* Glycine protects proximal renal tubules from hypoxic injury, however the mechanism of protection is unknown (1). To follow tubular metabolism, proximal tubules were isolated from rat kidney cortex, purified on a Percoll gradient and incubated with 5 mM [2-^{13}C] glycine under various conditions. High resolution ^{13}C NMR studies of PCA extracts of the tubules were obtained at 7.0 and 11.7 T. The results were correlated with measurements of LDH release to assess tubular integrity. Rapid conversion of glycine into serine was observed forming [2-^{13}C], [3-^{13}C] and [2,3-^{13}C] serine via both major metabolic pathways, viz serine hydroxymethyltransferase (SHMT) and the glycine cleavage complex (GC). Relative concentrations of [2-^{13}C]glycine and unlabelled glycine were determined from the ratio of single to double labeled serine. This technique enables the contribution of de novo glycine synthesis to be assessed. Reduced single labeled serine was observed when unlabeled glycine was removed from the preparation steps so that de novo synthesis became the only source of unlabeled glycine. De novo formation of glycine was stimulated by addition of unlabelled serine (reversal of SHMT) and by reoxygenation after hypoxia, suggesting proteolysis and/or peptide cleavage. Inhibition of GC by cysteamine resulted in the formation of only [2-^{13}C] serine. Hypoxia also resulted only in [2-^{13}C]serine formation, indicating complete GC inhibition with incomplete SHMT inhibition. These preliminary results support the suggestion [1] that protection by glycine protection of proximal tubules occurs with little or no metabolism of glycine itself. In addition, the demonstration that proteolysis is occurring during reoxygenation despite the presence of glycine suggests that, at least during this phase of injury, continuing protection by glycine is not by inhibition of proteolysis. (1) WEINBERG JM, DAVIS JA, ABARZUA M, RAJAN T: *J Clin Invest* 80:1446–1454, 1987

Immunohistochemical localization of chromogranin to glomerular peripolar cells of newborn sheep. *D. Alcorn, J. McCausland, and G.B. Ryan, Department of Anatomy, University of Melbourne, Parkville, Victoria, Australia.* Glomerular peripolar cells (PPCs) form a cuff around the origin of the tuft at the junction between parietal and visceral podocytic epithelium. One surface of PPCs is attached to Bowman's capsular basement membrane, the other is directly exposed to the urinary space. PPCs have been described in a wide range of species; they are most obvious in newborn lamb kidney. Several proteins (including albumin, neuron specific enolase, transthyretin) have been immunohistochemically localized to PPCs of newborn lamb kidney, suggesting that PPCs may be resorbing proteins from Bowman's space. However, in newborn lamb kidney, PPC cytoplasm appears highly synthetic with multiple secretory granules and evidence of regulative exocytosis into the urinary space. The aim of the present study was to examine PPCs for the presence of chromogranin, a secretory protein common to a variety of cells secreting peptides by the regulated pathway. Kidney tissue from newborn lamb kidney was fixed in Bouin's fluid and processed through to paraffin sections. Sections were immunostained with monoclonal mouse anti-human chromogranin A antiserum (Dako) at a range of concentrations using a modified peroxidase-anti-peroxidase technique. Chromogranin was specifically localized to PPCs. Glomeruli, tubules, and blood vessels showed no staining. The immunohistochemical localization of chromogranin to PPCs of the newborn lamb kidney provides new evidence for their role as a regulative secretory cell. The functions of the secretory product(s) of PPCs await clarification.

Glomerular charge selectivity: The differential processing of dextran sulfate and DEAE-dextran. *W.D. Comper, M. Tay, Y. Adal, E.F. Glasgow, and L. Pratt, Biochemistry and Anatomy Departments,*

Monash University, Clayton, Victoria, Australia. We have previously established (Zamparo and Comper: *Biophys Chem* 38:167, 1990) that the Deen-Brenner biophysical model for glomerular charge selectivity cannot hold. The aim of this study was to examine the processes that give rise to the differential fractional clearance of dextran sulfate and DEAE dextran. Studies have been performed with the isolated perfused rat kidneys which exhibit nearly normal charge selectivity as found in vivo. The sensitivity of charge selectivity to cycloheximide (Tay et al: *AJP* 260:F549, 1991), electron microscopical analysis, autoradiographical analysis, post-perfusion glomeruli concentration levels of the dextran transport probes, the kinetics of glomerular turnover of the various dextran transport probes and their binding to isolated glomerular preparations indicate that the [^{3}H]dextran sulfate but not [^{3}H]dextran, is actively endocytosed by endothelial cells (mesangial cells may also participate) and is disgorged back into the blood capillary space in a matter of minutes. This bypass route can account for the retarded movement of the glomerular transport of dextran sulfate compared to dextran. In the case of the facilitated transport of [^{3}H]DEAE it appears that this occurs, not through a cell-mediated process, but by it binding to the glomerular basement membrane and other glomerular components and altering the size selectivity of the membrane.

Ischemia lowers kidney osmoprotectant levels. *P. Sizeland, S. Chambers, M. Lever, L. Bason, R. Robson, Departments of Nephrology, Infectious Diseases and Biochemistry, Christchurch Hospital, Christchurch, New Zealand.* This study was carried out to investigate the effect of an acute ischemic injury on the renal osmoprotectant system. NZW rats were used, with the vascular pedicle of one kidney being ligated for 60 minutes. Inner medullary samples of both kidneys were then analyzed for the osmoprotectants glycine betaine(GB), myoinositol(MI), sorbitol(S) and glycerophosphocholine (GPC). Concentrations (mmol/kg tissue) are shown in the table (mean SEM, $N = 9$):

	GB	MI	GPC	S
Ischemic	4.2 (0.7)[a]	3.7 (0.4)[a]	6.4 (2.0)[a]	0.7 (0.2)
Non-ischemic	7.7 (0.3)	5.7 (0.9)	9.4 (1.9)	0.8 (0.1)

[a] $P < 0.01$, vs. non-ischaemic kidney

Ischemia significantly decreased the inner medullary concentrations of GB, MI, and S compared with non-ischemic controls. Perturbation of the osmoprotectant system may be important in the etiology of acute tubular necrosis, and the polyuria which characterizes the recovery from this.

Vasoactive intestinal peptide (VIP) inhibits sodium uptake in rat primary proximal tubular cell culture. *M.A. Lonergan, S. Aglibut, and M.J. Field, Department of Medicine, University of Sydney, Concord Hospital, N.S.W., Australia.* VIP has been proposed to play a role in the regulation of renal sodium (Na) excretion. It has recently been demonstrated to be present in peripolar cells, where it is ideally placed to modify renal tubular transport. We therefore examined the effect of VIP on Na uptake by rat proximal tubular epithelium. Primary cultures of proximal tubular cells from normal Sprague-Dawley rats were studied at confluence. After dispersion in dispase, cells were washed and incubated in low sodium buffer, at pH 7.4 for thirty minutes. Subsequently 4 groups were studied, characterized by the addition of ouabain (100 μM) alone as control, ouabain + VIP (1 μM), ouabain + amiloride (100 μM), and ouabain + amiloride + VIP. Following incubation for 15 minutes, aliquots were taken for protein estimation and cell counting. Na^{22} uptake was measured in each group at 1, 3 and 30 minutes by taking 20 μl of cell suspension and adding 70 μl of high sodium uptake buffer (Na 120) containing Na^{22} at 1 μCi per sample. The reaction was stopped at each time point by the addition of 1 ml of ice cold solution containing 149 mM LiCl, 0.5 mM Tris and 0.5 mM Hepes. 250 μl of the final solution containing cells was collected in a cell harvester onto filter paper and assayed for radioactivity in a beta counter. Results are expressed as cpm/mg protein and normalised to the mean of the sodium uptake in the control group at one minute in each study. At one minute, Na uptake in the presence of VIP was 66.2 ± 8.1% (SEM, $N = 6$) of the ouabain control. With amiloride alone, it was 44.6 ± 1.1% and with amiloride + VIP, it was 64.3 ± 8.9%. All experimental groups were significantly less than control ($P < 0.05$) but were not significantly different from each other. We conclude that VIP inhibits Na^{22} uptake in rat proximal tubular cells. The data are consistent with a mechanism of inhibition involving decreased activity of the Na-H exchanger in the apical cell membrane.

Anionic charge concentration of rat kidney glomeruli and glomerular basement membrane. *A.S.N. Lee, M. Tay, and W.D. Comper, Biochemistry Department, Monash University, Clayton, Victoria, Australia.* Estimates of levels of glomerular and glomerular basement membrane anion charge should serve as a useful quantitative markers for the integrity of the tissues in health and disease. We have developed a simple, rapid technique to measure this charge through the use of an exchange technique with radioisotopes $^{22}Na^+$ and $^{35}Cl^-$ at low ionic strengths in phosphate buffer. By this technique, normal glomeruli isolated from rat, have a measured charge concentration of 17.4 ± 3.7 pequiv. per glomerulus ($N = 20$). Perfused rat kidneys that lose approximately half of their glomerular [^{35}S]heparin sulfate content (due to oxygen radical damage) also exhibited a lower anion charge of 7.5 ± 1.6 pequiv. per glomerulus ($N = 5$). Glomerular basement membranes prepared from rat glomeruli by simply a sonication-centrifugation procedure had a charge concentration of 9.0 ± 1.0 mequiv. L^{-1} ($N = 4$) whereas membranes prepared by sonication, DNase and detergent treatment had a charge concentration of 10.8 ± 2.9 mequiv. L^{-1} ($N = 4$). These values are in good agreement with those obtained by others using titration techniques (Bray and Robinson: *Kidney Int* 25:527, 1984). We conclude that the exchange technique will provide a useful routine method by which glomerular and glomerular basement membrane net anion charge can be measured.

Does phosphate loading directly stimulate the parathyroids? *M. Cochran, B. Dilena, Department of Medicine and Department of Clinical Biochemistry, Flinders Medical Centre, SA, Australia.* It is widely accepted that the enhanced renal excretion of phosphate that follows parathyroid stimulation is secondary to changes in plasma calcium and/or $1,25(OH)D_2$, rather than phosphate itself. Given that PTH is such a powerful modulator of plasma phosphate, and that there is no other significant control system, it is surprising that phosphate has no direct effect at all. To test this, in 5 normal subjects, we administered Calcium Sandoz 1 g tds taken with meals, for six days. On the last three days, the subjects took, in addition, Phosphate Sandoz 1 g tds two hours after the calcium supplement. On the first day of the phosphate supplementation, the subjects omitted breakfast but took the calcium dose. Blood was sampled before and 60 minutes after the phosphate dose, which was taken on schedule. The sample was analyzed for total and ionized calcium, phosphate, intact PTH, and $1,25(OH)_2D$. The sampling was repeated one hour after the phosphate dose on the sixth day. Results showed that total and ionised calcium were not significantly altered during the study. Phosphate increased in all cases after the first dose but decreased in two subjects with chronic administration. The $1,25(OH)_2D$ showed no significant change. The PTH increased within the normal range in all cases following the acute phosphate dose, and in three cases after chronic administration, but decreased in the two subjects in whom the plasma phosphate fell. There was a highly significant relation between these two parameters ($P = 0.01$). The data support the idea that an acute phosphate load, and the associated elevation of the plasma level, can directly promote a parathyroid response.

Glomerular proteinuria: The involvement of glomerular cells and heparin sulfate. *Y. Adal, W.D. Comper, E.F. Glasgow, and M. Tay, Biochemistry and Anatomy Departments, Monash University, Clayton, Victoria, Australia.* Two distinct forms of proteinuria have been identified in an isolated perfused rat kidney (IPK) system. The first form is the perfusion time-dependent proteinuria that occurs in IPK which is accompanied by a release of glomerular heparin sulfate into the perfusate (Vassiliou et al, 1990) and a decrease in the total glomerular anion charge concentration. (Note that biophysical studies have demonstrated that while albumin is negatively charged, $Z = -19$) it would not be influenced by a direct charge effect (Coulombic repulsion) exerted by the GBM heparin sulfate (Zamparo and Comper, 1990)). A second, higher level of proteinuria can be induced by the presence of cycloheximide in the perfusate. This proteinuria is accompanied by no

change in the size selectivity nor glomerular anion charge concentration which would indicate that heparin sulfate charge is not involved in this particular type of albumin processing. Further, normal perfused kidneys accumulate high resident levels of glomerular [^{3}H] albumin during the perfusion. This glomerular albumin accumulation is markedly reduced when the perfusion is performed in cycloheximide. Isolated glomeruli from rat kidneys are also able to bind significant quantities of [^{3}H] albumin in vitro. Electron microscopical studies with gold-albumin have demonstrated the presence of vesicles containing albumin in endothelial cells after a two minute perfusion period. The results so far would be consistent with the hypothesis that some albumin is processed by glomerular cells through binding and an active uptake and exocytosis mechanism. VASSILIOU, P, TAY M and COMPER WD: *Biochem Int* 19:1241, 1989; Zamparo, Q, and Comper WD: *Biophys Chem* 38:167, 1990

The effect of interleukin 1 on glomerular sclerosis. *H.Y. Wang, Z.H. Zhao, Nephrology Center, Beijing Medical University, Beijing, China.* Interleukin 1 (IL-1) is a pleiotropic factor, eliciting a broad set of immunologic and inflammatory events. We have previously shown that mesangial cells (MSc) produced IL-1 like factors has the effect of stimulating MSc proliferation. To evaluate the role of IL-1 on glomerulosclerosis (GS), we observed IL-1 and TGF-β mRNA expression (Northern blot) during the development of GS and cellular matrix accumulation (collagen IV, CoIV, laminin, LN, fibronetin, FN, by ^{35}S incorporation and collagen assay) in ATS model. Also, the effect of rIL-1 β on stimulating cultured MSc expressing TGF-β mRNA and producing cellular matrix was studied. Our results showed: (1) During the development of GS in ATS rat, the expression of IL-1 and TGF-β increased (2- to 3-fold more than normal control) at day 7 combining with the increasing of CoIV (29.39 ± 2.40 vs. 2.64 ± 0.89), LN (20.46 ± 1.53 vs. 2.93 ± 0.18), and FN, (17.55 ± 1.05 vs. 4.91 ± 0.77), but not at day when GS was identified morphologically and matrix accumulation was continued. (2) rIL-1β stimulated TGF-β mRNA gene expression and promoted ^{35}S incorporation (1586 ± 282 vs. 700 ± 109), collagen synthesis (6.81 ± 0.70 vs. 4.20 ± 0.68) in subcultured MSc. These explorations of both experiment animal and cellular studies indicate that IL-1 plays an important role in the development of GS.

Involvement of activated interstitial leukocytes in disruption of Bowman's capsule and crescent progression in experimental anti-GBM disease. *H.Y. Lan, D.J. Nikolic-Paterson, R.C. Atkins, Department of Nephrology, Monash Medical Centre, Clayton, Victoria, Australia.* We have investigated whether interstitial leukocytes could induce glomerular damage by causing disruption of Bowman's capsule (BC) in experimental anti-GBM disease. Disease was induced in inbred Sprague-Dawley rats by administration of rabbit anti-GBM serum 5 days after immunization with normal rabbit IgG, with groups of 4 animals sacrificed at days 3, 7, 14, 21, and 28. Leukocytic infiltration was analyzed by monoclonal antibody (Mab) labeling of cryostat sections. Periglomerular macrophage and T cell infiltration was evident at day 3. Activated (IL-2R$^+$) mononuclear cells (MNC) were first seen in the hilar area at day 7, and were then found in focal accumulations in the periglomerular area from day 14. Disruption of BC was first evident at day 14; all animals at days 14 to 28 exhibited BC rupture, while 11/12 animals displayed crescent formation (25–74%). Analysis of 200 glomeruli per animal on PAS-stained paraffin sections found that disruption of BC occurred in both the presence (48–74%) and absence (26–52%) of crescent formation. However, disruption of BC was invariably associated with sites of focal periglomerular MNC infiltration, whereas BC remained intact where periglomerular infiltration was mild. Mab labeling revealed that focal MNC infiltrates associated with BC rupture contained IL-2R$^+$ cells and macrophages. Fibrous organization of cellular crescents and the presence of IL-2R$^+$ cells within crescents occurred only when BC was ruptured. In summary, this study has demonstrated that BC disruption occurred in both the absence and presence of cellular crescents, and that disruption of BC only occurred in tight association with focal periglomerular infiltrates of activated MNC. In glomeruli with crescent formation, disruption of BC appeared to be an essential step in progressive fibrosis of cellular crescents. Disruption of BC by interstitial leucocytes, via a DTH-type reaction, may be an important mechanism of glomerular damage.

Lipid peroxidation (LP) in passive heymann's nephritis (PHN). *T.J. Neale, P.P. Ojha, J. Witzum, H. Pocewski, M. Exner, D. Kerjaschki, Department of Medicine, Wellington School of Medicine; Institute of Pathology, Vienna, Austria, and Department of Medicine, UCSD, La Jolla, California, USA.* Our previous studies have shown that the rat glomerular epithelial cell (GEC) expresses the NADPH-oxido-reductase enzyme component, cytochrome b558 and that this is up-regulated after GEC activation by anti-Fx1A antibody in PHN. Cytochrome functional activity, assessed as H_2O_2 production, was specifically histochemically revealed ultrastructurally by cerium capture within proteinuric glomeruli. The current study sought to determine if LP occurred in PHN as a consequence of this local release of reactive oxygen species (ROS). The LP markers malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) were specifically identified within the glomeruli of rats with proteinuric PHN using monoclonal antibody IF and immunoblotting. Immunogold EM localized MDA to GEC structures (particularly cytoplasmic vesicles and cell membranes), the glomerular basement membrane (GBM), the subepithelial space, and to areas in relationship to immune deposits. Autoradiography of immunoprecipitates of tritium-labeled GBM from proteinuric rats using antisera against extracellular matrix proteins showed that type IV collagen was a ligand for MDA. These findings are consistent with the hypothesis that ROS-induced glomerular injury in PHN is mediated by LP, and the close temporal correlation with the onset of proteinuria suggests a causal role for LP in glomerular permselectivity alteration.

Total numbers of glomeruli and individual glomerular cell types in the normal rat kidney. *J.F. Bertram, M.C. Soosaipillai, S.D. Ricardo, and G.B. Ryan, Department of Anatomy, University of Melbourne, Parkville, Victoria, Australia.* Various renal disorders are associated with changes in glomerular and glomerular cell number. In the present study, new unbiased stereological counting methods (Gundersen et al: *APMIS* 96:857–881, 1988) were used to estimate total numbers of glomeruli and individual glomerular cell types in normal rats. Unlike traditional stereological methods, these new methods do not require assumptions of glomerular or nuclear shape, size distribution or orientation, and thereby provide unbiased estimates. The kidneys of seven female Sprague-Dawley rats weighing 215 ± 16 g (mean ± SD) were perfused with 4% paraformaldehyde and 1% glutaraldehyde in phosphate buffer and tissue was processed for embedding in either glycolmethacrylate (light microscopy, LM) or epon/araldite (transmission electron microscopy, TEM). Total glomerular number was estimated using a physical disector/fractionator combination at the LM level, the total number of cells per average glomerulus was estimated using an optical disector/Cavalieri combination at the LM level, and numbers of individual cell types were estimated using TEM physical disectors. The normal rat kidney was found to contain 31,764 ± 3,667 glomeruli. An average glomerulus contained 674 ± 129 cells, of which 181 ± 53 were epithelial cells (podocytes), 248 ± 53 were endothelial cells, and 245 ± 45 were mesangial cells. An average renal corpuscle contained 117 ± 27 parietal epithelial cells. These estimates generally agree with those previously obtained using traditional counting methods such as serial section reconstruction, maceration, or biased stereological methods. The efficacy of the present counting methods are remarkable: on average, following sectioning and staining, less than 6.5 hours was needed to obtain estimates for a single animal, with coefficients of variation (standard deviation expressed as a percentage of the mean) ranging from 10% to 30%. These data provide a baseline for experimental studies of glomerular disease.

Urinary platelet factor 4 (PF4) and mesangial IgA glomerulonephritis (IgAGN). *Koji Taira, Tim Hewitson, and Priscilla Kincaid-Smith, Department of Nephrology, Royal Melbourne Hospital, Victoria, Australia.* The aim of this study was to determine if platelets and the specific platelet release protein PF4 are associated with glomerular injury in IgAGN. Urine samples from patients with IgAGN were examined by ELISA to determine the concentration of PF4 ([PF4]; N = 51) and by immunoperoxidase (IP) staining for the presence of urine sediment platelets (N = 18). Renal biopsy tissue taken within 24 hours of urine sampling was stained by IP for glomerular platelets (N = 39). Elevated urinary [PF4] (≥0.07 ng/ml) was associated with the presence of active glomerular crescents ($P < 0.005$) and fibrin deposition ($P <$ 0.01). [PF4] was higher in patients with crescents ($P < 0.05$), and

glomerular fibrin deposition ($P < 0.05$) than those without. Likewise, the presence of glomerular platelets and urine platelets was associated with glomerular crescent formation ($P < 0.02$, $P < 0.05$, respectively), although there was no such association with fibrin deposition. There was no relationship between urine [PF4], urine platelets or glomerular platelets and the presence of either global sclerosis or focal and segmental hyalinosis and sclerosis. There was a strong correlation between elevated urine [PF4] and the presence of glomerular PF4 staining ($P < 0.0001$) and urine sediment platelets ($P < 0.005$). These results suggest that an assay for urine [PF4] may be a useful prognostic indicator of severe glomerular injury in patients with IgAGN.

Electron microscopy of glomerular changes in experimental pre-eclampsia. *A. Hennessy, A.F. Phippard, P.D. Kirwan, D.M. Painter, J.S. Horvath, Department of Renal Medicine and Anatomical Pathology, Royal Prince Alfred Hospital, Camperdown, Sydney, NSW, Australia.* Rational management of pre-eclampsia (PE), a syndrome of hypertension and proteinuria arising de novo in pregnancy and reversed by delivery, has been confounded by two problems: lack of diagnostic criteria and uncertain pathogenetic mechanisms. To test the proposition that placental ischemia causes pre-eclampsia, we have conducted studies in the chronically-instrumented baboon (*Papio hamadryas*). During late pregnancy hypertension and proteinuria are experimentally induced by reduction in placental perfusion. Renal histology performed at the time of delivery and at twelve weeks post-partum has demonstrated the characteristic glomerular lesion of PE by light microscopy (LM). The aim of the present study was to further characterize by electron microscopy (EM) the glomerular pathology associated with this animal model of PE. Open renal cortical wedge biopsy was performed at the time of delivery. Tissue blocks 1 mm × 1 mm were immediately fixed in 2.5% glutaraldehyde and processed in a routine fashion for EM. They were then examined with toluene blue staining for abnormal glomeruli. These glomeruli were then scanned by transmission electron microscopy TEM (Phillips). The same animals had contralateral renal biopsies at three months post-partum. EM studies provided ultrastructural definition of glomerular swelling and capillary changes identified by LM. Contributing abnormalities were endothelial cell swelling and disruption with resultant glomerular capillary occlusion. Additional changes were subendothelial fibrin deposits with early reduplication of the basement membrane as well as mesangial interposition. Some fibrinoid and dense deposits were seen in the glomerular basement membranes and mesangial areas. No further pathology indicative of inflammatory change was identified. The post-partum biopsies had regressed. These sequential EM findings support the proposition that placental hypoperfusion leads to pre-eclampsia.

A quantitative analysis of renal arteriosclerosis in diabetic nephropathy: Clinicopathological correlations. *Ian R. Fraser, Kathy M. Nicholls, Priscilla S. Kincaid-Smith, Department of Nephrology, Royal Melbourne Hospital, Melbourne, Victoria, Australia.* A computer aided image-analyzer was used to determine an index of arteriosclerosis in the renal biopsies of diabetics with nephropathy. All blood vessels judged to be sectioned end on were measured. The blood vessel wall and total blood vessel surface area measured, and the two were expressed as a ratio. The mean ratio for a patient was taken as the index of arteriosclerosis (IA). A total of 62 diabetics and 17 controls (15 immediate transplant biopsies and 2 graft nephrectomies for carcinoma) were measured by the one blinded observer. A minimum of 15 blood vessels were measured, with a mean coefficient of variance of 10.7%. Patients characteristics are listed below.

	No.	Age	Sex (M:F)	Creatinine	Proteinuria
Diabetic	62	46 (20–71)	35:17	0.11 mmol/liter	0.94 g/day
Control	17	42 (26–64)	7:10	–	

All diabetics had a mean urinary albumin excretion (UAE) greater than 30 μg/min. Forty-two patients had glomerular filtration rate (GFR) estimated by technetium labeled DTPA nuclear scan. The diabetics had a significantly greater index of arteriosclerosis (IA) when compared to controls (0.794 vs. 0.751 $P < 0.001$). There was a significant correlation between the IA and estimates of renal function; GFR ($r = -0.38$, $P < 0.001$), creatinine ($r = 0.40$, $P < 0.004$). There was no correlation between IA and the following: systolic or diastolic blood pressure, age urinary protein excretion, duration of diabetes, type of diabetes, serum cholesterol and past of present cigarette use. We describe a method of quantifying arteriosclerosis in renal biopsies. There is a significant correlation between the severity of arteriosclerosis and renal function, suggesting extraglomerular arteriolar disease may be an important determinant of renal function in diabetes.

Sub-thrombotic dose of thrombin produces thrombi in the glomeruli of cyclosporine (CsA)-treated rabbits. *P. Faraco. T. Hewitson, P. Kincaid-Smith, Department of Nephrology, Royal Melbourne Hospital, Victoria, Australia.* CsA has been associated with glomerular thrombosis and the mechanisms involved are not known. Previously, we reported that infusion of sub-thrombotic doses of endotoxin in CsA-treated rabbits produces massive deposition of fibrin in the glomeruli. To investigate if inflammatory mediators stimulated by endotoxin are important factors involved in the model above, we infused CsA-treated rabbits with sub-thrombotic doses of endotoxin-free thrombin. Twenty NZW rabbits were divided in four groups. The animals received CsA (25 mg/kg/day) or diluent for 10 days and were infused with thrombin (1 HIH unit/kg/min) or saline on day 11. After infusion they were sacrificed, kidneys removed and processed for light and electron microscopy. Control group received CsA diluent and saline. CsA group received CsA for 10 days and saline. Thrombin group received diluent for 10 days and thrombin. CsA + thrombin group received CsA and thrombin. No fibrin deposition was found in control and CsA groups. We found 3.7% glomeruli with fibrin in the thrombin group and 76.6% glomeruli with fibrin in CsA + thrombin group ($P < 0.001$). No glomerular thrombi were observed in the thrombin group but in group CsA + thrombin 47.3% glomeruli contained thrombi. These results are very similar to those observed for CsA-treated rabbits infused with endotoxin. We concluded that the deposition of fibrin in the glomeruli of rabbits treated with CsA and infused with endotoxin is not due to the interaction between CsA and inflammatory mediators as it has been hypothesized before.

Lysosomal iron (FE), reactive oxygen species (ROS) and puromycin nephrosis (PAN). *B.J. Nankivell, C. Tay, G. Mill, R.A. Boadle, D.C.H. Harris, Department Renal Medicine and EM Unit, Westmead Hospital, NSW, Australia.* ROS have been implicated in the pathophysiology of puromycin nephrosis. As Fe catalyses ROS production and has been found in other models of renal disease, this study examined for the presence of Fe and its possible relationship to ROS and proteinuria in male Wistar rats given PAN (10 mg/100 g body wt i.v.) and saline controls. At the time of peak proteinuria (day 12) and recovery (day 33), Fe was examined by atomic absorption spectroscopy and EM energy dispersive analysis, transferrin (TF) by radial immunodiffusion and ROS by malondialdehyde (MDA).

	Peak ($N = 14$)	Recovery ($N = 19$)	$P <$
Proteinuria *mg/24 hrs*	277.4 ± 19.2[b]	15.1 ± 0.9	0.001
Urine Fe *μg/24 hrs*	22.4 ± 2.1[b]	2.2 ± 0.3[a]	0.001
Urine TF *mg/24 hrs*	34.9 ± 3.4[b]	0.44 ± 0.11	0.001
Plasma MDA *nmol/liter*	23.9 ± 5.1[b]	9.8 ± 0.7	0.01
Tissue MDA *nmol/mg protein*	0.55 ± 0.04	0.72 ± 0.09	NS
Lysosomal Fe *wt %*[c]	331.8 ± 65.1[b]	120.5 ± 33.6	0.01

[a] $P < 0.05$, [b] $P < 0.01$ vs. controls, [c] control = 26.5 ± 13.7

The numbers of Fe containing lysosomes/tubule correlated with urinary Fe ($r = 0.91$, $P < 0.001$) and also with urine TF and proteinuria. In conclusion, iron accumulates in proximal tubule lysosomes during the proteinuric phase of PAN nephrosis, probably gains access to the cell across the brush border membrane and appears to turn over rapidly.

Iron (FE) depletion and reactive oxygen species (ROS) in the remnant kidney. *B.J. Nankivell, R.A. Boadle, C. Tay, G.K. Mill, D.C.H. Harris,*

Department of Renal Medicine, Westmead Hospital, NSW, Australia. Fe accumulates in RK proximal tubular lysosomes, and by catalysis of ROS may be involved in disease progression. To determine the significance of lysosomal Fe accumulation, the effect of Fe depletion (by dietary restriction ± venesection) was examined in 5 groups of partially nephrectomized (⅝) male Wistar rats. Groups 1–3 were pairfed a diet containing adequate cysteine and methionine containing sulphydryl (SH) groups, which scavenge ROS and groups 4–5 were pairfed a diet deficient in SH groups, for 6 months. GFR was assessed by Tc^{99} DTPA clearance, Fe by energy dispersive analysis, and ROS by MDA production and glutathione.

Gp	Diet Fe *mg/kg*	GFR *ml/min/kg body wt.*	Proteinuria *mg/24 hrs*	Lysosomal Fe *mean wt%*	Hb *g/liter*
1	100	2.45 ± 0.30	40.1 ± 9.4	197.4 ± 70.3	150 ± 6
2	0	2.05 ± 0.43	56.3 ± 18.7	36.3 ± 20.9[a]	121 ± 5[a]
3	0 + v	2.70 ± 0.34	37.5 ± 10.9	10.9 ± 6.1[a]	128 ± 9[a]
4	250	2.70 ± 0.30	87.4 ± 15.2	51.0 ± 17.67	157 ± 5
5	0 + v	2.10 ± 0.47	209.2 ± 21.7[b]	32.7 ± 11.7	123 ± 9[b]

Mean ± SEM; [a] $P < 0.05$ vs. gp1, [b] $P < 0.01$ vs. gp4

In Fe replete rats, lysosomal Fe correlated with proteinuria ($r = 0.63$, $P = 0.014$) and MDA production ($r = 0.50$). In SH-deficient rats, Fe depletion was associated with increased tissue MDA (0.74 ± 0.05 vs. 0.57 ± 0.06 nmol/mg protein, gp 5 vs. 4, $P < 0.05$) and reduced total glutathione (164.5 ± 5.1 vs. 108.7 ± 6.7 μmol/mg protein, $P < 0.05$), and tissue MDA correlated with proteinuria ($r = 0.62$, $P = 0.016$). In summary, in RK Fe depletion reduces lysosomal Fe without protecting renal function, and exacerbates injury when ROS are not scavenged.

Proximal tubular lysosomal iron (FE) in the remnant kidney (RK). *B.J. Nankivell, R.A. Boadle, D.C.H. Harris, Department of Renal Medicine, Westmead Hospital, NSW, Australia.* Fe accumulates in proximal tubule lysosomes of several models of chronic renal failure. To examine the significance of proximal tubule lysosomal Fe in RK, a morphological study was performed in male Wistar rats after 5/6 nephrectomy ($N = 17$) vs. controls (N, $N = 8$). After in vivo perfusion with Karnovsky's solution, cortical sections were examined by conventional EM for type and size of lysosomes in proximal tubules and cell damage (scored 0–4) and energy dispersive analysis for Fe (quantified by the Hall method).

Lysosomes		RK	*N*	*P* <
Primary	%	41.2	23.5}	chi^2 0.001
Secondary	%	29.6	47.9}	chi^2 0.001
Phagolysosomes	%	29.2	28.6	NS
Number/tubule[a]		8.42 ± 0.41	11.97 ± 0.42	0.001
Area μm^{2a}		0.39 ± 0.02	0.34 ± 0.01	0.001
Fe *wt %*[a]		220.7 ± 65.5	58.8 ± 27.9	0.05
Cell damage score[a]		2.94 ± 0.04	0.68 ± 0.03	0.001

[a] Mean ± SEM

In RK rats, proteinuria correlated directly with the number of Fe containing lysosomes/proximal tubule ($r = 0.56$, $P = 0.03$) and with mean lysosomal area ($r = 0.66$, $P < 0.01$). Calculated lysosomal volume increased by 41% in RK. Tubular cell damage correlated directly with lysosomal Fe content ($r = 0.41$, $P = 0.005$). In summary, RK have more primary lysosomes, and larger digestive secondary lysosomes containing increased amounts of Fe, abnormalities which are associated with cell damage and may be induced by proteinuria.

Glomerular volume in non-insulin dependent diabetes mellitus. *Ian R. Fraser, Kathy M. Nicholls, Priscilla S. Kincaid-Smith, Department of Nephrology, Royal Melbourne Hospital, Melbourne, Victoria, Australia.* We quantified the light microscopic changes in a group of patients with insulin dependent diabetes mellitus (IDDM), ($N = 20$), and non-insulin dependent diabetes mellitus (NIDDM), ($N = 19$), and compared them to cadaveric donors, matched for age ($N = 28$). The renal donors were separated into 2 groups, to match the ages of the diabetic groups. All diabetics had a mean urinary albumin excretion (UAE) greater than 30 μg/min. A quantitative analysis was performed blindly—on sections stained with periodic acid Schiff's reagent (PAS), using point counting. The following parameters measured: (1) mean glomerular volume (MGV), (2) volume fraction of capillary lumina (VvC), and (3) – volume fraction of PAS positive material (VvP). MGV for both NIDDM (1.57 Mμ3 vs. 0.88 $P < 0.001$) and IDDM (1.22 vs. 0.62 $P < 0.001$) groups were significantly greater than controls on point counting. There was no difference between NIDDM and IDDM for either VvC or VvP. MGV was significantly greater in the NIDDM when compared to the IDDM ($P < 0.05$). There was a significant correlation between age and MGV in the control group ($r = 0.55$, $P = 0.003$). These results suggest that the glomerular hypertrophy seen in IDDM also occurs in NIDDM, and that the nature of the enlargement is the same. The difference in MGV between the two diabetic groups can be explained, in part, by the effect of ageing on glomerular volume, as demonstrated in the control group.